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Poster

674. Adult Neurogenesis

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 674.01/A1

Topic: A.01. Neurogenesis and Gliogenesis

Support: HRF-201512-015

Title: Combined Zinc plus N-acetyl-L-cysteine supplement increases adult hippocampal neurogenesis

Authors: *D. HONG[1], B. CHOI[1], B. LEE[1], S. LEE[1], A. KHO[1], S. LEE[1], J. JEONG[1], M. SOHN[2], S. SUH[1];
[1]Hallym Univ., Chuncheon, Korea, Republic of; [2]Inha Univ., Incheon, Korea, Republic of

Abstract: Adult hippocampal neurogenesis (AHN) occurs in the subgranular zone (SGZ) of the dentate gyrus (DG), where high levels of vesicular zinc are localized. Zinc is an essential trace element involved in numerous biological functions, including DNA synthesis, hormone control, enzymatic activity, and cell proliferation. Recent studies, including those from our lab, have demonstrated that Zn^{2+} plays an important role in regulating AHN. In addition, several antioxidants such as flavonoids, vitamin E, curcumin, and N-acetyl-L-cysteine (NAC) increase neurogenesis in the rodent brain. Thus, in the present study, we evaluated the effect of Zn^{2+}-NAC complex ($Zn(NAC)_2$) on hippocampal neurogenesis in adult mice. C57BL/6 male mice (8 weeks old), were divided into four groups: Vehicle, $ZnCl_2$, NAC and $Zn(NAC)_2$ treated groups. The mice were given intraperitoneal injections of $ZnCl_2$ (5 mg/kg), NAC (25 mg/kg) or $Zn(NAC)_2$ (30 mg/kg) once daily for 2 weeks. Vehicle-treated mice were injected on the same schedule with saline. To confirm the proliferative identity of cells, BrdU was intraperitoneally injected twice daily for 4 consecutive days before sacrifice. Neurogenesis was evaluated by immunohistochemistry for BrdU, Ki67 and doublecortin (DCX). Mice receiving 5 mg/kg $ZnCl_2$ alone, showed no differences in the number of progenitor cells and neuroblasts, compared with vehicle-treated mice. However, NAC-treated mice showed a significant increase in the number of progenitor cells and neuroblasts. Furthermore, $Zn(NAC)_2$-treated mice showed an even greater increase in the number of progenitor cells and neuroblasts. The present study demonstrates the additive and synergistic effects of $Zn(NAC)_2$ on progenitor cell proliferation and neuroblast production, respectively. These findings suggest that $Zn(NAC)_2$ may promote the regenerative capacity of the adult brain as a potent enhancer of hippocampal neurogenesis. Keywords : zinc, n-acetly-l-cysteine, $Zn(NAC)_2$, neurogenesis, hippocampus

Disclosures: **D. Hong:** None. **B. Choi:** None. **B. Lee:** None. **S. Lee:** None. **A. Kho:** None. **S. Lee:** None. **J. Jeong:** None. **M. Sohn:** None. **S. Suh:** None.

Poster

674. Adult Neurogenesis

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 674.02/A2

Topic: A.01. Neurogenesis and Gliogenesis

Support: HK RGC GRF Grant No. 777313

Title: Overexpressed APPL2 result a depressive- and anxiety- like behaviors, which associated with impaired hippocampal neurogenesis

Authors: *G. CHONG[1], X. CHEN[1], A. XU[2], J. SHEN[1];
[1]Sch. of Chinese Medicine, LKS Fac. of Med., The Univ. of Hong Kong, Hong Kong SAR, Hong Kong; [2]Dept. of Med., The Univ. of Hong Kong, Hong Kong, SAR, Hong Kong

Abstract: Adult hippocampal neurogenesis plays important roles in animals' mood and cognitive regulation. Impaired growth and differentiation of neural stem cells (NSCs) usually associates with many types of neurodegenerative or psychiatric disease. Relationship between neurogenesis and depression disorder is nowadays most popularized studied not only because that depression is nowadays a common and serious psychiatric disorder affects people's daily life, but it also because that increased neurogenesis can truly buffer the stress induced mood disorder. Daptor protein, phosphotyrosine interaction, PH domain and leucine zipper containing (APPL) appears to play a critical role in cell growth and metabolism modulation. In two isoforms of APPLs protein, APPL1 was reported to associate with the regulation of insulin sensitivity by facilitating the binding of IRS1/2 to the insulin receptor. APPL2, the other isoform of APPLs, participate in the glucose uptake in skeletal muscle. However, whether APPLs participate with the neurogenesis regulation remains unexplored. In this study, we obtained APPL2-Tg mice with overexpressed APPL2 protein. We found APPL2-Tg mice presented a spontaneous depressive- and anxiety- like symptoms though different types of behavioral tests. Furthermore, our immunofluorescence indicated that overexpressed APPL2 limited the production of new neurons and the proliferation of the radial glia-like stem cells (RGLs) in hippocampus. The *in vivo* clone analysis also indicated that APPL2 overexpression decreased the intermediate progenitor cell (IPC) fate choice of the RGLs. And we found the maturation process of the young neurons was also delayed in APPL2-Tg mice. Combine these lines of evidence together; we can conclude that APPL2 might acts in the animal mood regulation and antidepressant behaviors that associated with its functions in neurogenic modulation.
Keywords: Depression, APPL2, Neurogenesis, RGLs.

Disclosures: **G. Chong:** None. **X. Chen:** None. **A. Xu:** None. **J. Shen:** None.

Poster

674. Adult Neurogenesis

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 674.03/A3

Topic: A.03. Stem Cells and Reprogramming

Support: EPITARGET

Title: Directed differentiation of neural progenitors in dentate gyrus in naïve and epileptic rats.

Authors: ***E. MELIN**[1], X. XIAN[2,3], N.-B. WOODS[2,3], T. RAMOS-MORENO[3,4], M. KOKAIA[4];
[1]Lunds Universitet, LUND, Sweden; [2]Div. of Mol. Med. and Gene Therapy, Lund, Sweden; [3]Stem Cell Ctr., Lund, Sweden; [4]Epilepsy Ctr., Lund, Sweden

Abstract: Neural progenitors (**NP**) in the adult *dentate gyrus* (**DG**) continuously produce new functional granule cells. It is well known that epileptic environment in the hippocampus promotes neurogenesis in an early stage of the disease and could be considered to be a different niche than in the healthy hippocampus. Here we study *in vivo* directed differentiation of the neural progenitors in the adult DG in naïve and epileptic animals. We generate *status epilepticus* (**SE**) animals by injecting a sub cutaneous dose of kainic acid, and target neural progenitors with a retroviral vector coding for Ascl1 and/or Dlx2 transcription factors, both being expressed by GABAergic cells during different stages of development. Our results show that different glia and neuronal populations are generated from the infected NP in both naïve and epileptic environments. For example, after retroviral infection coding for Ascl1 in epileptic rats, immunohistochemical analysis reveals that one majority cell population is positive for neuro/glial antigen 2 (NG2), a marker for oligodendrocytic progenitor cells (OPCs) and another population expresses NeuN, a marker for mature neurons. These results shows for the first time that overexpression of a single transcription factor in an epileptic environment is able to direct differentiation of NP, which has implications for treatment of pharmacoresistant epilepsy, memory formation and neurological affections.

Disclosures: **E. Melin:** None. **X. Xian:** None. **N. Woods:** None. **T. Ramos-Moreno:** None. **M. Kokaia:** None.

Poster

674. Adult Neurogenesis

Location: Halls B-H

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Program#/Poster#: 674.04/A4

Topic: A.01. Neurogenesis and Gliogenesis

Support: European 7th Framework Programme NeuroKine Grant 31672

Title: Efficient isolation of viable primary neural cells from adult murine brain tissue based on a novel automated tissue dissociation protocol

Authors: H. ZHANG[1], S. REIß[1], S. TOMIUK[1], S. RÜBERG[1], R. FEKETE[2], M. JUNGBLUT[1], *A. BOSIO[1];
[1]Miltenyi Biotec, Bergisch Gladbach, Germany; [2]Fluidigm Corp., South San Francisco, CA

Abstract: Tissue dissociation and preparation of single-cell suspensions with high cell viability and a minimum of cell debris is the prerequisite for cellular analysis, cell culture, and cell separation. As dissociation of adult brain requires sophisticated mechanical and enzymatic treatment to successfully disaggregate the tightly connected neural cells, cell analyses is often restricted to embryonic or neonatal murine tissue. We have set up a new technology for dissociation of neonatal brain by combining automated mechanical dissociation using the gentleMACS™ Octo Dissociator (Miltenyi Biotec) with an optimized enzymatic treatment. To extend the analyses to adult neural cells we have further optimized the automated dissociation process and included a novel protocol for removal of debris, which is crucial for successful cell isolation and culture. The new process increased the number of viable cells and yielded 3-5x10E6 total living cells per adult mouse brain. Subsequently, astrocytes, oligodendrocytes, neurons, microglia, or endothelial cells were separated from dissociated adult murine brain tissue by magnetic cell sorting (MACS). Astrocytes were isolated using Anti-ACSA-2 (astrocyte cell surface antigen-2) MicroBeads. The process yielded 4.0x10E5 ± 0.7x10E5 astrocytes with a viability of 69.1 ± 10.3% and a purity of 93.9 ± 5%. In case of neurons, non-target cells were depleted using a mixture of non-neuronal cell markers. 2.0x10E5 ± 0.25x10E5 neurons with a viability rate of 72.0 ± 7.1% and a purity of 92.2 ± 1.1% were acquired per adult mouse brain. Likewise, 1.2x10E5 ± 0.25x10E5 oligodendrocytes with a viability of 76.7 ± 10.3% and a purity of 90.0 ± 6.7% were separated using Anti-O4 MicroBeads, mouse and rat. Purified Microglia were gained by using CD11b MicroBeads, mouse or CD11b/c MicroBeads, rat, respectively. A purity of 97-99% and a viability of 94.7% ± 4.2% was obtained. In case of astrocytes, highly purified cells were further subjected to a single-cell mRNA sequencing analysis in order to characterize neonatal and adult astrocyte diversity. Therefore, the C1 Single-Cell Auto Prep System (Fluidigm) was used for single-cell capturing of highly purified neonatal and adult astrocytes (98.5-99%) and preparation of cDNA libraries. Single-cell transcriptome analyses

revealed a highly diverse expression profile of neonatal and adult astrocytes with distinct subgroups within each population. In summary, we present a novel standardized technology to generate highly purified and viable adult neural cells that extends the analysis from neonatal to adult murine brain tissue and facilitates sophisticated cellular and molecular analyses.

Disclosures: **H. Zhang:** 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; This work was supported by the EU seventh framework programme NeuroKine (no 31672). **S. Reiß:** None. **S. Tomiuk:** None. **S. Rüberg:** None. **R. Fekete:** None. **M. Jungblut:** None. **A. Bosio:** None.

Poster

674. Adult Neurogenesis

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 674.05/A5

Topic: A.01. Neurogenesis and Gliogenesis

Title: Region-specific transcript profiles of the lncRNA Sox2ot in adult rat brain: correlation with Sox2 enhancers and Sox2 expression.

Authors: *D. A. CARTER[1], S. YULE[2];
[2]Sch. of Biosci., [1]Cardiff Univ., Cardiff, United Kingdom

Abstract: Sox2 is a transcription factor gene with numerous, partially defined, roles in neural development. Recent studies have identified multiple novel enhancer sequences that span 200kb of the Sox2 locus, and act to confer brain region-, and embryonic stage-specific expression (and activity) of Sox2 (Okamoto et al [2015] Develop. Growth Differ. 57, 24-39). We are investigating the molecular mechanisms that underlie an unusual pattern of adult Sox2 expression in the adult ventral diencephalon, including neurons of the suprachiasmatic nucleus (SCN). One class of potential regulators are long non-coding (lnc) RNAs; in addition to the string of genomic enhancers across the Sox2 locus, this region is also spanned by a multi-exon lncRNA, Sox2ot (Sox2-overlapping transcript), that may contribute to cell-type-specific Sox2 expression. In the current study, we investigated the expression of Sox2ot isoforms in the SCN, and correlated transcripts with Sox2 enhancer sequences that confer specificity for distinct brain regions, including the ventral diencephalon ('U6' enhancer). In order to identify potential SCN-specific expression of Sox2ot isoforms, expression was compared with olfactory bulb (OB) and somatosensory cortex (COR). Sox2ot sequences were amplified using PCR primers directed against known (rat/ mouse/human) exons/EST sequences of both Sox2ot, and also Sox2dot, a group of isoforms that include distal (more than 500kb upstream of Sox2) exons. We found that

both Sox2ot and Sox2dot transcripts are abundantly expressed in rat brain, and the relative abundance of specific isoforms is brain region-specific. For Sox2dot, transcripts are expressed across OB/SCN/COR but the pattern of isoform expression is markedly distinct in COR, compared with OB and SCN. This result is consistent with a similar (immature) transcript profile in OB and SCN. However, for Sox2ot, the transcript profiles are similar in SCN and COR, and distinct from OB. These findings concur with the well-known cell-type specificity of lncRNA expression, and evidence distinct Sox2ot transcript profiles across OB/SCN/COR. Expressed Sox2ot/dot exons closely align with Okamoto enhancers (3/4, and 2/5, respectively), and a common 5' Sox2dot exon also aligns with a VISTA forebrain enhancer (hs192). However, we found no evidence that Sox2ot/dot incorporate U6 sequence, and could not amplify U6 independently of Sox2ot/dot exons. Our results indicate two classes of Sox2 enhancer: (1) transcribed within Sox2ot/dot in adult brain, and (2) non-transcribed. Differential use of these enhancer/ lncRNA(+/-) units may contribute to cell-type specific Sox2 expression, including that in the adult SCN.

Disclosures: D.A. Carter: None. **S. Yule:** None.

Poster

674. Adult Neurogenesis

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Program#/Poster#: 674.06/A6

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant 3T32DA007287-18S1

Other Support: John S. Dunn Foundation

Title: Unique regional and sex-dependent properties of adult endogenous neural stem cells to chronic alcohol and cocaine co-adminstration.

Authors: *E. L. MCGRATH[1], J. GAO[2], T. DUNN[2], A. GRANT[2], J. ALLENDE-LABASTIDA[2], K. DINELEY[3], B. KAPHALIA[4], K. CUNNINGHAM[5], P. WU[2]; [1]UTMB, Galveston, TX; [2]Neurosci. and Cell Biol., [3]Neurol., [4]Pathology, [5]Pharmacol. and Toxicology, Univ. of Texas Med. Br., Galveston, TX

Abstract: Cocaine and alcohol are two of the most commonly co-absued substances, and the third most fatal drug combination. Efforts in drug addiction research primarily focus on preventing or stoping abuse, however little work is being done to reverse brain damage incurred by chronic drug abuse. Neural stem cells are a promising target to stimulate brain recovery,

however little is known about the effect of long term co-abuse of alcohol and cocaine on this cell population. Additionally, sex differences in NSC behavior following chronic drug abuse have yet to be evaluated. This is the first study to evaluate regional and sex differences of endogenous adult neural stem cells to chronic treatment with alcohol and cocaine. We sought to elucidate the response of adult endogenous NSCs to chronic alcohol and cocaine treatment using a inducible lineage tracing mouse model. Adult mice were randomly divided into 1 of 4 groups: control, cocaine, ethanol, or combination treatment. Daily i.p. injections of cocaine were administered and ethanol was provided in a complete nutrient liquid diet to appropriate groups. Brain tissue was analyzed for markers of NSC survival and differentiation. The subventricular zone of lateral ventricle (SVZ) subgranular zone of dentate gyrus (SGZ) and tanycyte layer of third ventricle (TL) were evaluated. KEY FINDINGS: We found that NSCs have a unique response to drug depending on the regional location and sex of the animal. Females had more robust decreases in neural stem cell (NSC) survival in SVZ compared to males. SGZ neurogenesis was reduced in combination group. The TL showed the greatest variation in astroglial marker (glial fibrillary acidic protein) among both sexes and treatment groups.

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

Poster

674. Adult Neurogenesis

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 674.07/A7

Topic: A.01. Neurogenesis and Gliogenesis

Support: Canadian Institutes of Health Research (CIHR)

Title: The effects of high-fat diet on adult neurogenesis in the hippocampus

Authors: ***F. NASRI**, M. WOJTOWICZ;
Dept. of Physiol., Univ. of Toronto, Toronto, ON, Canada

Abstract: High-fat diet (HFD) is associated with poor cognitive health and exacerbation of neurodegenerative diseases. Some of the effects of HFD have been attributed to impairments in neurogenesis and cognitive functions of the hippocampus, a brain area important for learning and memory. However, how the specific attributes of the diet such as the diet length and fat percentage affect hippocampal neurogenesis is not fully understood. This study examines the effects of a 4-week or 8-week period of HFD (45% of total energy contributed by saturated fatty

acids) on the levels of adult hippocampal neurogenesis. 12 male Long-Evans rats (4 weeks old) were used for each of the two studies. In the 4-week HFD study, the animals were fed either HFD (n=6) or regular chow diet (n=6) for 4 weeks, and for the 8-week HFD study, the animals were fed either HFD (n=6) or regular chow diet (n=6) for 8 weeks. The food consumption and caloric intake of the animals were measured every week over the study period. The HFD-fed animals consumed less food but their caloric intake was higher than the chow-fed animals (as each gram of the HFD was more energy-dense than one gram of regular chow). This suggests that the HFD-fed animals may have been trying to compensate for the energy-dense HFD by eating less food although the results do not show a complete compensation. Furthermore, only the HFD-fed animals in the 8-week experiment underwent a significantly higher weight gain compared to the chow-fed animals. After the end of the diet period the animals were sacrificed. Hippocampal sections were stained for doublecortin (DCX; marker for young neurons) and NeuN (marker for mature neurons) to examine the levels of neuronal differentiation and maturation respectively. Cell numbers expressing DCX and NeuN were quantified per section in the dorsal, medial and ventral regions of the hippocampus or per dentate gyrus. There was no significant difference in DCX or NeuN expression between the HFD- and chow-fed animals in either of the two studies. This lack of effect may be due to these specific HFD periods (i.e. 4 weeks and 8 weeks) not being long enough to show changes in neurogenesis. Longer periods of HFD may result in higher levels of inflammation, higher percentages of weight gain and/or lower levels of physical activity that could in turn result in significantly reduced levels of neurogenesis. Additionally, it may take a longer time after the end of the diet period for changes in neurogenesis to show. Therefore, an increased period of high-fat diet exposure or prolongation of the monitoring period after the exposure may be required to show effects.

Disclosures: F. Nasri: None. **M. Wojtowicz:** None.

Poster

674. Adult Neurogenesis

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 674.08/A8

Topic: A.01. Neurogenesis and Gliogenesis

Title: Matrix metalloproteinase-12 regulates the establishment of the subventricular zone neural stem cell niche

Authors: *X. SHAN, Q. YANG, H. A. COLOGNATO;
Pharmacol. Sci., Stony Brook Univ., Stony Brook, NY

Abstract: The subventricular zone (SVZ) houses the largest neural stem cell niche in the mammalian brain. In this niche, molecular cues from special cellular and extracellular structures tightly regulate stem cell proliferation and differentiation. Apical surfaces of SVZ neural stem cells are encircled by multiciliated ependymal cells, forming a pinwheel-shaped cellular arrangement. Ependymal cells arise postnatally from neural stem cells, and the movement of their motile cilia directs the flow of cerebrospinal fluid, ensuring proper neurogenesis. The planar cell polarity of ependymal cells coordinates the orientation of this ciliary movement.
The adult apical SVZ contains a distinctive extracellular matrix (ECM), which we have recently found to develop concurrently with the formation of niche pinwheels. While this niche ECM is diffuse at birth, by adulthood it becomes highly polarized to discrete regions in niche pinwheel centers, which we have termed “ECM hubs”.
Since a dramatic remodeling of both cells and ECM occurs during postnatal SVZ development, we hypothesized that matrix metalloproteinases (MMPs), a family of extracellular endopeptidases that regulate ECM spatial organization in many tissues, may play a role in niche reorganization. Here we report that MMP-12 expression in particular is highly upregulated during ependymal cell maturation, and that MMP-12 knockout mice have an abnormal SVZ niche structure, characterized by aberrant numbers of stem cells per pinwheel, and reductions in the number and size of ECM hubs. Furthermore, the establishment of planar cell polarity in niche ependymal cells is significantly disrupted in MMP-12 knockout mice.
Surprisingly, we found that lentivirus- or neonatal ventricle electroporation-mediated acute knockdown of MMP-12 impeded ciliogenesis in ependymal cells, which was not observed in MMP-12 knockout mice. We found that in MMP-12 knockout ependymal cells, a truncated MMP-12 mRNA is still expressed and can be translated into a shorter, intracellular isoform of MMP-12. Intracellular MMP-12 has been recently reported to act as a novel transcription factor that can enhance the transcription of IκBα, a negative regulator of NFκB signaling. As inappropriate NFκB pathway activation has recently been linked to a ciliogenic defect in ependymal cells, our data suggests a model in which aberrant NFκB activation upon MMP-12 knockdown leads to a ciliogenic defect in ependymal cells. Together, these findings indicate that both secreted and intracellular MMP-12 may regulate the establishment of cellular and extracellular structures of the SVZ neural stem cell niche.

Disclosures: **X. Shan:** None. **Q. Yang:** None. **H.A. Colognato:** None.

Poster

674. Adult Neurogenesis

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 674.09/A9

Topic: B.12. Glial Mechanisms

Support: of Chungnam National University (2015)

Title: Primary cilia modulate TLR4-mediated NF-kB signaling in hippocampal neurons

Authors: H. BAEK[1], J.-J. KIM[2], J. PARK[1], C.-S. KIM[1], *D. KIM[3];
[1]Physiol., [2]Anat., Chungnam Natl. Univ. Sch. of Med., Daejeon, Korea, Republic of; [3]Chungnam Natl. Univ., Daejeon, Korea, Republic of

Abstract: The primary cilium is an organelle that can act as a master regulator of cellular signaling. Despite the presence of primary cilia in hippocampal neurons, their function is not fully understood. Recent studies have demonstrated that the primary cilium influences interleukin (IL)-1β-induced NF-κB signaling, ultimately mediating the inflammatory response. We, therefore, investigated ciliary function and NF-κB signaling in lipopolysaccharide (LPS)-induced neuroinflammation in conjunction with ciliary length analysis. Primary ciliary length decreased in hippocampal pyramidal neurons after intracerebroventricular injection of LPS, whereas it increased in TLR4-/- mice. Next, to exclude the effects of microglial TLR4, we utilized HT22 hippocampal neuronal cells. LPS treatment decreased primary ciliary length, activated NF-κB signaling, and increased Cox2 and iNOS levels in HT22 hippocampal neurons. In contrast, silencing Kif3a, a key protein component of cilia, increased ciliary protein levels and suppressed NF-κB signaling and expression of inflammatory mediators. These data suggest that LPS-induced NF-κB signaling and inflammatory mediator expression are modulated by cilia, and that blockade of primary cilium formation by si_Kif3a regulates TLR4-induced NF-κB signaling. We propose that primary cilia are critical for regulating NF-κB signaling events in neuroinflammation and in the innate immune response.

Disclosures: H. Baek: None. **J. Kim:** None. **J. Park:** None. **C. Kim:** None. **D. Kim:** None.

Poster

674. Adult Neurogenesis

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 674.10/A10

Topic: A.01. Neurogenesis and Gliogenesis

Title: The atypical RhoGTPase Rnd2 is critical for newly generated neurons in the adult hippocampus

Authors: *T. KERLOCH[1], A. GORON[1], F. GUILLEMOT[2], N. D. ABROUS[1], E. PACARY[1];
[1]Neurogenesis and Physiopathology, Neurocentre Magendie, Bordeaux, France; [2]Div. of Mol. Neurobio., Natl. Inst. for Med. Res., London, United Kingdom

Abstract: Adult-born hippocampal neurons go through distinct developmental steps, from a dividing neurogenic progenitor to a synaptically integrated mature neuron and this requires an extensive and dynamic remodeling of the cytoskeleton at each step of the process. Nevertheless, few studies have explored the role of the RhoGTPases, which are well-known regulators of the actin cytoskeleton, in the regulation of adult hippocampal neurogenesis. In this context, we have focused our attention on relatively newly characterized members of this family: the Rnd proteins and in particular on Rnd2. Rnd2 plays a critical role during embryonic neurogenesis in the cerebral cortex and is expressed in the adult dentate gyrus. However the implication of Rnd2 in the process of adult hippocampal neurogenesis is still unknown.
To address this role, we have implemented two strategies of loss of function. First, we used retroviruses expressing Cre together with GFP to delete *Rnd2* expression in newborn neurons of the dentate gyrus of $Rnd2^{flox/flox}$ mice. With this strategy, we show that *Rnd2* deletion affects the survival of newborn neurons and induces an ectopic migration as well as an acceleration of the dendritic maturation in the surviving new neurons. Second, we have generated $NestinCreER^{T2}/Rnd2^{flox}/AI6$ mice to study the impact of *Rnd*2 deletion on the functions sustained by hippocampal adult-born neurons in particular spatial navigation and pattern separation. We are now analyzing these mice to confirm the effects observed with the retroviral strategy.
Our preliminary data reveal a novel function for Rnd2 in the adult brain and provide mechanistic insights into the critical aspect of adult neurogenesis regulation.

Disclosures: T. Kerloch: None. **A. Goron:** None. **F. Guillemot:** None. **N.D. Abrous:** None. **E. Pacary:** None.

Poster

674. Adult Neurogenesis

Location: Halls B-H

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Program#/Poster#: 674.11/A11

Topic: A.01. Neurogenesis and Gliogenesis

Support: CONACyT Grant 239516

Title: The expression of Gas1 is regulated by proneural factors Neurogenin (Ngn2) and NeuroD via distal E-boxes

Authors: *M. A. QUEZADA, J. SEGOVIA;
Physiol., CINVESTAV, Mexico, Mexico

Abstract: Gas1 is a membrane GPI-linked protein that inhibits cell proliferation by blocking the PI3K-Akt transduction signal when overexpressed. We have taken advantage of this

characteristic for pre-clinical gene therapy assays directed at treating glioblastoma. However, the physiological role of Gas1 is more complex than previously considered and genetic studies have indicated that it is an important regulator of central nervous system development acting as a co-receptor for Sonic Hedgehog (Shh) during the establishment of neuronal progenitor cells in spinal cord and granular cells of cerebellum. Recently we observed the expression of Gas1 in embryonic proliferating progenitor cells of cortex and hippocampus indicating that Gas1 fulfills a relevant function during brain development. On the other hand, although several studies have been published about Gas1 gene regulation little is known about the mechanisms that govern its pattern of expression during the development of the nervous system (specifically in neuronal cell linages) an aspect that is key, to understand the role of Gas1 in neurogenesis. As a first step to reach this objective we analyzed the promoter sequence of the mouse Gas1 gene by comparative genomics searching for transcription factor binding sites that could explain the expression pattern of Gas1 in the nervous tissue. Interestingly, the screening of the mouse Gas1 promoter revealed the presence of at least three highly conserved E-boxes with the consensus sequence CANNTG that can bind the proneural basic-helix loop helix (bHLH) transcription factors Neurogenin 2 (Ngn2) and NeuroD. These proneural factors are known to regulate different stages of neurogenesis, including the specification of neuronal progenitor cells, the differentiation and the establishment of neuronal subtypes. We cloned a fragment of the mouse Gas1 promoter and performed luciferase reporter assays in the N1E-115 neuroblastoma cell line. By deletion and mutagenesis assays we demonstrated that two distal E-boxes motifs, located -1699 and -1940 relative to the transcription start site of Gas1, mediate its activation by potentially binding to Ngn2, NeuroD and NeuroD2 proteins. This is the first time, to our knowledge, that a relationship between Gas1 and basic proneural signals that induce neuronal fate is observed.

Disclosures: **M.A. Quezada:** None. **J. Segovia:** None.

Poster

674. Adult Neurogenesis

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 674.12/A12

Topic: A.01. Neurogenesis and Gliogenesis

Title: mTORC1 regulates retinal development via the immune proteasome

Authors: *J.-H. CHOI;
KAIST, Taejon-City, Korea, Republic of

Abstract: The growth and development of mouse retina are regulated by cellular mechanisms that couple neurogenesis to the proliferation of retinal progenitor cells (RPCs). In this study,

mammalian target of rapamycin complex 1 (mTORC1) pathway was determined to be explored in connection with retinal development. We identified a regulatory role of mTORC1 in retinal development, showing that it facilitates both the synthesis and degradation of cyclin proteins to accelerate the cell cycle progression of RPCs and consequently the retinal neurogenesis. Interestingly, it has also revealed that the level of immune proteasome subunit β-9 (Psmb9) highly correlated with mTORC1 activity in developing mouse retina, and concomitant loss of Psmb9 decelerated cell cycle progression in *Tsc1*-deficient mouse RPCs and normalized retinal development. These results together support the hypothesis that mTORC1 may coordinates protein synthesis and degradation during retinal development process through a mechanism involved by immune proteasome.

Disclosures: J. Choi: None.

Poster

674. Adult Neurogenesis

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Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

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Topic: A.01. Neurogenesis and Gliogenesis

Support: NIDCR 1R03DE024783

Title: *In vitro* model for characterizing migration and differentiation of primary cranial and trunk neural crest cells isolated from Sox9-cre/EYFP mouse embryos

Authors: *M. R. REPLOGLE[1], K. R. SVOBODA[2], A. J. UDVADIA[1];
[1]Biol. Sci., [2]Joseph J. Zilber Sch. of Publ. Hlth., Univ. of Wisconsin - Milwaukee, Milwaukee, WI

Abstract: Neural crest cells (NCCs) are a transient population of embryonic stem-like cells that have the potential to differentiate into neurons and glia of the peripheral nervous system, as well as a variety of other non-neural derivatives including the skeletal elements of the vertebrate head. We are interested in investigating epigenetic modulation of genes regulating NCC migration and differentiation, and the potential disruption of this modulation by genetic and environmental insults. In order to obtain cell numbers sufficient for biochemical analyses of protein-protein and protein-gene interactions in developing NCCs we have optimized conditions for expanding and differentiating murine NCCs *in vitro*. Specifically we have isolated cranial and trunk NCCs from E9.5 *Sox9-cre/EYFP* mouse embryos. These cells can be expanded in culture for several passages while maintaining mesenchymal morphology, and expression of neural crest and stem cell-like markers. Using this *in vitro* model we have found that undifferentiated murine NCCs

stain positive for choline acetyltransferase, and thus have the potential to produce cholinergic signals prior to differentiation as observed previously in chick and quail. In addition, undifferentiated murine NCCs may also possess the capacity to respond to cholinergic signals based on their expression of nicotinic acetylcholine receptor subunits. Here we present a detailed characterization of these cholinergic properties, as well as the morphology and migratory behavior of *Sox9*-expressing NCCs in culture under proliferative conditions and during different stages of neuronal, glial, and chondrocytic differentiation.

Disclosures: **M.R. Replogle:** None. **K.R. Svoboda:** None. **A.J. Udvadia:** None.

Poster

674. Adult Neurogenesis

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 674.14/B1

Topic: A.01. Neurogenesis and Gliogenesis

Title: Expression of glycoprotein non-metastatic melanoma B in the developing rat brain

Authors: *S. YOKOYAMA;
Kanazawa Univ., Kanazawa, Japan

Abstract: The glycoprotein non-metastatic melanoma B (Gpnmb) is a type-I transmembrane protein that is produced by various types of normal cells including melanocytes, osteoclasts, osteoblasts, and dendritic cells in peripheral blood, as well as by various carcinoma cells. An increasing number of studies have described that Gpnmb is abundantly expressed in invasive glioblastomas, suggesting its contribution to tumor progression and metastasis. We have reported that Gpnmb is produced by macrophages and microglia in the normal and central nervous system of adult rats (Huang *et al.*, Brain and Behavior 2, 85-96, 2012). In this study, we further examined whether Gpnmb was expressed in the developing rat brain. Gpnmb mRNA was detected by reverse transcription-polymerase chain reaction analysis in postnatal day 1 (P1) brain at roughly the same level as in the adult brain. Immunoperoxidase staining detected intense Gpnmb-immunoreactivity (IR) in choroid plexus, leptomeninges, and ependyma through the postnatal period. In the cerebral cortex, Gpnmb-positive cells were distributed diffusely in all layers at P1; some of the cells were positive for OX42, a microglia/macrophage marker. After P7, Gpnmb-positive cells became more prevalent in cortical layers II-IV, as shown in adult rats. In addition to cell bodies, the Gpnmb-IR was also detectable in process-like structures. In the hippocampus, Gpnmb-IR became more intense in the dentate gyrus after P7. In the cerebellum, as in the cerebrum, Gpnmb-positive cells were observed in all layers at P1. At P15 and P30, Gpnmb-IR was steadily detectable in Bergmann glial cells, with cell bodies adjacent to Purkinje

cells, as previously demonstrated in adult rats. Additionally, in the both cerebrum and cerebellum, Gpnmb-positive cells were detected in the white matter and inner layers most frequently at P7 and P15, suggesting involvement in myelin formation. These results suggest that Gpnmb plays multiple roles in the development of postnatal rat brain.

Disclosures: S. Yokoyama: None.

Poster

674. Adult Neurogenesis

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 674.15/B2

Topic: A.03. Stem Cells and Reprogramming

Support: NSFC Grant 31171359

NSFC Grant 81560457

Title: Nek2A phosphorylates and stabilizes SuFu: a new strategy of Gli2-Hedgehog signaling regulatory

Authors: *S. LUO[1], Y. WANG[2], Y. LI[2], G. HU[2], H. RAO[3], Q. LU[4];
[2]Ctr. for Exptl. Med., [1]The First Affiliated Hospital, Nanchang Univ., Jiangxi, China; [3]Dept. of Mol. Med., Univ. of Texas Hlth. Sci. Ctr., San Antonio, TX; [4]Dept. of Biostatistics & Epidemiology, Sch. of Publ. Health, Nanchang Univ., Jiangxi, China

Abstract: The Hedgehog (Hh) signaling pathway plays pivotal roles in normal embryonic development and postnatal homeostasis. Suppressor of Fused (SuFu), one of the key negative regulators of Hh signaling and Gli activities, is indispensable in vertebrates, as ablation of SuFu in mouse embryos leads to widespread activation of the Hh pathway and causes early embryonic lethality with cephalic and neural defects. Despite the central importance of SuFu in the Hh pathway, little is known about its regulation. Here, we performed a GAL4-based yeast two-hybrid screen using human SuFu as bait, and identified NIMA-related expressed kinase 2A (Nek2A) as a new SuFu-interacting protein, which was also confirmed by Glutathione-S-transferase pull-down and co-immunoprecipitation assays. Intriguingly, Nek2A is found to stabilize SuFu at least partly depending on its kinase activity, thereby triggering phosphorylation of the SuFu protein. Moreover, the phosphorylated SuFu inhibits the nuclear localization and transcriptional activity of Gli2. These findings reveal a new mechanism of mammalian SuFu regulation, and offers novel insights into Hh signaling regulation in development and human disease.

Disclosures: **S. Luo:** None. **Y. Wang:** None. **Y. Li:** None. **G. Hu:** None. **H. Rao:** None. **Q. Lu:** None.

Poster

675. Peripheral Nervous System Regeneration

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 675.01/B3

Topic: A.04. Transplantation and Regeneration

Support: CIHR RES0023156

University Hospital Foundation RES0029278

NSERC RES0007116

Title: Bone morphogenetic protein-7 and the development of the peripheral pain pathway

Authors: ***C. A. WEBBER**[1], T. FRIEDMAN[2], P. SABIRI[2], T. JOY[2], V. ZOUVELOU[4], D. GRAF[3];
[1]Div. of Anat., [3]Dent., [2]Univ. of Alberta, Edmonton, AB, Canada; [4]Univ. Hosp. Agia Sofia, Athens, Greece

Abstract: Neuropathic pain (NP) is a priority health issue that affects up to 10% of all adults worldwide. Medical interventions are often inadequate and can lead to drug tolerance or addiction. An understanding of the development of the pain pathways may provide essential clues to develop novel treatments for NP. Bone Morphogenetic Proteins (BMPs) are evolutionarily conserved, secreted, highly regulated signaling molecules with pleiotropic functions. Loss of Bmp7 affects multiple neural crest-derived craniofacial structures including a significantly delayed outgrowth of the trigeminal nerve. As Bmp7 has been implicated in neural development and regeneration of the peripheral nerves, we sought to investigate whether loss of Bmp7 affects development and function of peripheral sensory neurons. Bmp7-lacZ reporter mice revealed that in E9.5 embryos *Bmp7* is expressed in newly formed neural crest cells. At E11.5 *Bmp7* was detected in a mottled pattern in migrating dorsal root ganglion neurons (DRGN) suggesting it is expressed in a subpopulation. Lineage tracing for neural crest-derived cells using the Rosa26lacZ allele and wnt1-Cre revealed that at E15.5 *Bmp7*-deficient mice have noticeably fewer spinal nerve axons and a decrease in size of DRGs. To assess potential functional consequences (and to overcome perinatal lethality), we deleted *Bmp7* in all neural crest-derived cells using a conditional *Bmp7* allele (*Bmp7fl*) and wnt1-Cre. Behavioral analysis (Von Frey filament test) in adult *Bmp7*fl:wnt1Cre mutant mice revealed a significant allodynia. Histological

analysis of corresponding DRGs demonstrated a decrease in calcitonin gene related protein (CGRP)-expressing neurons found in a subpopulation of both Aδ (primarily sharp pain) and C fibers (dull ache/inflammatory pain) (12.8% ± 0.6 and 21.7% ± 0.2% of CGRP expressing DRGN in control and *Bmp7*fl:wnt1Cre mutant mice respectively ($p<0.05$)). Conversely in C-fibers there was an increase in isolectin B4 (IB4)-expressing DRGN from 33.0% ± 0.07% in control mice to 45.7% ± 0.16 in the *Bmp7*fl:wnt1Cre mutant mice respectively ($p < 0.05$). In summary, our data reveal for the first time a role of Bmp signaling in pain fiber sub-specification and regulation of physiological pain responses. In future work we will explore this lead to identify molecular networks involved in pain fiber sub-specification, which might lead to novel targets for therapeutic intervention in neuropathic pain.

Disclosures: C.A. Webber: None. **T. Friedman:** None. **P. Sabiri:** None. **T. Joy:** None. **V. Zouvelou:** None. **D. Graf:** None.

Poster

675. Peripheral Nervous System Regeneration

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 675.02/B4

Topic: A.04. Transplantation and Regeneration

Support: NIH Grant NS057190

Title: The effect of electrical stimulation on muscle reinnervation and axon elongation in a mouse model of Val66Met

Authors: *C. MCGREGOR[1], A. ENGLISH[2];
[1]Cell Biol., Emory Univ., Decatur, GA; [2]Cell Biol., Emory Univ., Atlanta, GA

Abstract: A single nucleotide polymorphism (SNP), Val66Met, results in abnormal regulated release of brain derived neurotrophic factor (BDNF) from neurons. The presence of this SNP could impact the translational capacity of activity-dependent treatments which enhance nerve regeneration after injury through a BDNF-dependent mechanism. We tested the effects of 1 hour of 20 Hz ES on muscle reinnervation in wild type (WT) C578/6 mice and mice of the same background strain in which the native BDNF gene was replaced by one containing the Val66Met SNP. Sciatic nerves were cut and repaired with end-to-end anastomosis, and 4 weeks later motor unit number estimation (MUNE) was performed using EMG recordings from lateral gastrocnemius. Motor endplate reoccupation was evaluated using histological sections of lateral and medial gastrocnemius. To measure axon regeneration, Val66Met mice were crossed with SLICK (Single neuron Labeling with Inducible Cre mediated Knockout) mice, which express

yellow fluorescent protein (YFP) in a subset of neurons, and nerve transection was repaired with acellular allografts from WT mice. Axon profile lengths of YFP+ neurons were measured two weeks post-repair. From all three outcome measures, our preliminary data indicate that untreated mice expressing the Val66Met SNP have superior regeneration than WT mice after injury, but that treatment with ES does not further enhance their regeneration. The enhancement of regeneration in the untreated Val66Met mice may be a result of compensatory mechanisms other than BDNF that are unaffected by ES.

Disclosures: C. McGregor: None. **A. English:** None.

Poster

675. Peripheral Nervous System Regeneration

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 675.03/B5

Topic: A.04. Transplantation and Regeneration

Support: AIHS CRIO team grant

CIHR operating grant

Title: Realizing the potential of adult human skin-derived Schwann cells for transplantation therapy

Authors: *J. A. STRATTON[1,2], R. KUMAR[2], P. SHAH[2,1], M. STYKEL[2], R. MIDHA[1,3], J. BIERNASKIE[2];
[1]Hotchkiss Brain Inst., Calgary, AB, Canada; [2]Dept of Comparative Biol. and Exptl. Med., [3]Dept Clin. Neurosciences, Univ. of Calgary, Calgary, AB, Canada

Abstract: Nerve injury causes significant long-term disability. With this in mind, we believe that supplementing the injured nerve with autologous Schwann cells (SCs), a glia cell type that can be harvested directly from injured patients, and that has potential to rejuvenate the injury environment, will improve functional outcomes. Although promising, Schwann cell collection procedures for autologous transplant, currently under clinical trial, require invasive surgery for collection, causing additional discomfort. As such, for over a decade now several laboratories have been developing protocols to derive SCs from the easily accessible skin. Using rodent skin-derived SCs (SkSCs), it has been shown that SkSCs can improve anatomical measures, ultimately supporting improved behavioral outcomes following CNS and PNS injury. Because most of the convincing research pertaining to SkSC potential has been demonstrated using neonatal rodent cells, it is paramount that similar experiments are perform using adult human

SkSCs before they can be seriously considered as a replacement for current treatment options. Here we describe the purification and characterization of SCs from adult human skin of 4 male donors (27-46 years old). Within 2 weeks of isolating and culturing adherent mixed skin cells in serum-free SC media, colonies of bipolar shaped cells were sporadically detectable. Within 2-4 weeks of growth, we selected these colonies using cloning cylinders, and re-plated these colonies. By 5 weeks, we obtained 3-5 million purified SCs - a cell number appropriate for transplantation in the clinic. Using a battery of Schwann cell lineage-specific markers, we demonstrated that SkSCs are phenotypically indistinguishable from nerve-derived SCs (nSCs). Namely, SC associated genes were highly expressed in both nSCs and SkSC cultures compared to dermal fibroblast cultures. This included genes such as SOX10, SOX9, AP2A1, CDH19, EGR1, ETV5, PAX3, SOX2, CX32, DHH, NECL4, NFATC4, POU3F1, S100B and YY1. Using immunocytochemistry we demonstrated that the percentage of cells in nSC and SkSC cultures expressing Sox10 were 89.3±6.3% and 77.3±6.2%, respectively (P=0.2). Following transplantation into nerve injury in immune-deficient mice, the majority of transplanted SCs maintained Sox10 immunoreactivity in both nSCs and SkSC transplant conditions, and also expressed the promyelinating factor, POU3F1, in 60.85±2.21% and 50.85±1.87% of cells, respectively (P=0.09). In addition, subsets of these cells were associated with myelin (MBP, Pzero or fluoromyelin+), aligned on neurofilament+ axons. Such findings suggest that SkSCs should be considered for clinical application.

Disclosures: **J.A. Stratton:** None. **R. Kumar:** None. **P. Shah:** None. **M. Stykel:** None. **R. Midha:** None. **J. Biernaskie:** None.

Poster

675. Peripheral Nervous System Regeneration

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 675.04/B6

Topic: A.04. Transplantation and Regeneration

Support: NIH Grant NS057190

NIH Grant NS087915

Title: Androgen receptor signaling is required for activity-dependent somatic motoneuron axon regeneration

Authors: ***P. J. WARD**[1], J. D. ZAJAC[2], A. W. ENGLISH, 30306[1];
[1]Emory Univ. Dept. of Cell Biol., Atlanta, GA; [2]Univ. of Melbourne, Heidelberg, Australia

Abstract: Systemic treatment with flutamide, an anti-androgen, blocks the enhancement of axon regeneration produced either by brief electrical stimulation (ES) or moderate treadmill exercise following peripheral nerve injury. The specific cellular target sites of the required androgen receptor (AR) signaling are unknown. Here, we used mouse genetics to investigate whether the requirement for AR signaling was in somatic motoneurons whose axons are regenerating. Two weeks following sciatic nerve transection and repair by end-to-end anastomosis, sciatic nerves were soaked in fluorescent retrograde tracer applied 4 mm distal to the repair. In SLICK (Single neuron Labeling with Inducible Cre mediated Knockout) mice, which express YFP in a subset of motoneurons, one hour of 20 Hz ES applied just prior to nerve repair resulted in a greater number of retrogradely-labeled and double-labeled (YFP+ and retrograde labeled) motoneurons than SLICK mice that underwent transection and repair alone (untreated). When SLICK:AR floxed mice are tamoxifen treated, the DNA binding portion of the AR is knocked out selectively in the YFP+ motoneurons. In male mice with this inducible AR knock-out, ES failed to promote axon regeneration in the knock-out (YFP+) motoneurons (fewer double-labeled motoneurons), but enhanced axon regeneration in the wild type (YFP-) motoneurons. In females, sciatic nerves in these same genotypes were cut and repaired using non-fluorescent nerve grafts. Lengths of YFP+ regenerating axon profiles were measured in confocal images two weeks later. Brief ES enhanced the median lengths of YFP+ axons in SLICK:AR floxed mice with motoneuron-specific AR signaling knock-out. Activity-enhanced axon regeneration of motoneurons is dependent on the transcriptional cascades resulting from AR signaling in motoneurons in males but not females. ES does not enhance motor axon regeneration via motoneuron ARs in a transcription-dependent manner in females.

Disclosures: P.J. Ward: None. **J.D. Zajac:** None. **A.W. English:** None.

Poster

675. Peripheral Nervous System Regeneration

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 675.05/B7

Topic: A.04. Transplantation and Regeneration

Title: A novel local FK506 delivery system for the treatment of peripheral nerve injury.

Authors: *K. TAJDARAN[1,2], T. GORDON[3], G. H. BORSCHEL[1,2];
[1]Univ. of Toronto/Sickkids Hosp., Toronto, ON, Canada; [2]Inst. of Biomaterial and Biomed. Engin., Toronto, ON, Canada; [3]SickKids Res. Inst., Toronto, ON, Canada

Abstract: Purpose: Many challenges exist in improving outcomes following peripheral nerve injuries, specifically in cases with delayed nerve repair and with large nerve defects. This study

focused on investigating a new method to improve axon regeneration after surgical repair of a severely injured nerve. FK506, an FDA approved immunosuppressant, promotes functional recovery and reinnervation following peripheral nerve injury. However, FK506 has not been widely adopted for treating nerve injuries because the systemically delivered drug causes undesirable immunosuppression. We investigated a novel local delivery system for FK506 which utilizes fibrin gel as a biodegradable drug reservoir that could be placed at a site of nerve injury.
Methods: FK506 was incorporated into fibrin gel in solubilized, particulated and poly(lactic-*co*-glycolic) acid (PLGA) microspheres-encapsulated forms. In order to analyze the effectiveness of the delivery systems in enhancing nerve regeneration, a rat nerve transection model was used, where the proximal tibial nerve stump was cross-sutured to the distal stump of a cut common peroneal nerve. Rats in the negative control groups either did not receive any delivery system treatment or received fibrin gel with empty microspheres (without any FK506). The experimental groups included rats treated with fibrin gel loaded with solubilized, particulated, or PLGA microspheres encapsulated FK506. 3 weeks after repair, nerve regeneration was assessed using retrograde labeling and collecting nerve samples 7 mm distal to the repair site for histomorphometric analysis.
Results: Rats in experimental groups receiving FK506-loaded microspheres and the particulate form of FK506 doubled the number of motoneurons regenerating their axons after injury and allowing *all* the tibial motoneurons to regenerate their axons successfully. The number of the motor and sensory neurons that regenerated their axons for the FK506 microspheres treated group and the particulated FK506 treated group were significantly higher than the number in all the groups, including the solubilized FK506 treated group and negative control groups. Histomorphometric analysis indicated increased numbers of myelinated axons following particulated FK506 and FK506 microspheres treatment compared to the native control groups.
Conclusion: The local application of FK506 via our proposed delivery systems resulted in excellent axon regeneration while preventing the toxicity of systemic FK506 that has prevented clinicians from using FK506 routinely for treating severe cases of peripheral nerve injuries.

Disclosures: **K. Tajdaran:** None. **T. Gordon:** None. **G.H. Borschel:** None.

Poster

675. Peripheral Nervous System Regeneration

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 675.06/B8

Topic: A.04. Transplantation and Regeneration

Support: NS057190

Title: Luminopsin mediated attenuation of exercise induced functional improvements after sciatic nerve injury

Authors: *P. B. JAISWAL[1], J. K. TUNG[2], R. E. GROSS[2], A. W. ENGLISH[1];
[1]Cell Biol., [2]Neurosurg., Emory Univ., Atlanta, GA

Abstract: Inhibitory luminopsins (iLMOs) integrate opto- and chemo-genetic approaches and allow for cell-type specific inhibition of neuronal activity. iLMOs are fusion proteins with a light- emitting, Renilla luciferase (RLuc), and a light-sensing inhibitory opsin, Halorhodopsin (NpHR). A small molecule substrate, coelenterazine (CTZ), binds to RLuc and is converted into photons that activate the NpHR channel to hyperpolarize cells expressing the iLMOs. Moderate daily exercise following peripheral nerve injuries (PNI) results in enhanced axon regeneration. An interval treadmill-training (IT) regimen is effective in female mice. We hypothesized that iLMO mediated inhibition of motoneuronal activity during exercise would attenuate the associated improvements in functional outcomes after sciatic nerve injury. Utilizing Cre-lox technology and viral vector delivery methods we expressed iLMOs in motoneurons. Unilateral intramuscular injections of AAV9-EF1a-DIO-iLMO2 (~8.5x1013 vg/ml) were made in young female ChAT-Cre mice. Four-six weeks were allowed for retrograde viral transduction of motoneurons after which a unilateral sciatic nerve transection (Tx) and repair was performed. Animals were randomized into four groups: IT with CTZ treatment, CTZ treatment only, IT only, and untreated (UT). Three days after Tx-repair, IT and/ or CTZ treatments were administered every day, five days a week for two weeks. Evoked M- response recovery was measured at three weeks post Tx-repair. The maximal M- response amplitude recorded from the reinnervated muscles of the IT only group was significantly greater than that in UT mice, confirming that this exercise regimen was effective in promoting axon regeneration and muscle reinnervation. Inhibiting motoneuronal activity during exercise in the IT+CTZ group resulted in a significant attenuation of the maximum M-response in this group, compared to the IT only group. The maximum M response amplitude in the CTZ only group was smaller than that found in UT mice at this survival time, indicating that inhibiting the activity of axotomized motoneurons impeded the regeneration of their axons. We conclude that neuronal activity may be required for successful motor axon regeneration and its enhancement by exercise.

Disclosures: **P.B. Jaiswal:** None. **J.K. Tung:** None. **R.E. Gross:** None. **A.W. English:** None.

Poster

675. Peripheral Nervous System Regeneration

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 675.07/B9

Topic: A.04. Transplantation and Regeneration

Support: CIHR

Title: Rat slow and fast nerves regenerate into appropriate endoneurial tubes to reinnervate tibialis anterior (TA) muscles after common peroneal (CP) nerve transection and surgical repair but, size-dependent branching occurs more distally within intramuscular sheaths

Authors: *T. GORDON[1], J. E. TOTOSY DE ZEPETNEK[2];
[1]Dept of Surgery, Div. of Plastic Reconstructive Surgery, Hosp. for Sick Children, Toronto, ON, Canada; [2]Univ. of Alberta, Edmonton, AB, Canada

Abstract: Muscle fibers innervated by one nerve, a muscle unit (MU), may be identified with periodic acid staining (PAS) after repetitive on-off fatiguing tetanic contractions of isolated and physiologically characterised MUs. MU muscle fibers are located within defined MU territories that normally occupy 21 $\pm$ 2 % (n=20) of the cross-sectional area of TA muscles. These MU territories, that link the outermost muscle fibers, provide an indirect image of the extent of intramuscular nerve branching (Rafuse and Gordon, *J Neurophysiol 75:282-297, 1996*). MU contractile forces and muscle fiber numbers increase with size of their innervating nerves in normal and reinnervated muscles (Gordon and Stein, *J Neurophysiol 48: 1175-1190, 1982*; Totosy de Zepetnek and Gordon, *J Neurophysiol 67: 1385-1403, 1992*). It follows that extent of nerve branching, as measured by the size of MU territories, and electrophysiological parameters of motor nerve size must also correlate. Here we asked 1) Is this prediction accurate in normal and reinnervated TA muscles? 2) If and how does the pattern of MU nerve branching within intramuscular nerve sheaths change after the known random axon regeneration into distal nerve stumps after CP nerve transection and surgical repair?; and 3) Is the normal preference of slow nerves to reinnervate muscle fibers in the deep portions of skeletal muscles, slow muscle fiber regionalization, demonstrated during nerve regeneration? The answers were 1) Yes. MU territories that were significantly reduced in size, demonstrated the same size-dependent relationship between the size of the motor nerve and the extent of the intramuscular branching of the nerve prior to formation of functional nerve-muscle contacts, as in normal TA muscles; 2) Yes. The pattern of single motor nerve branching *was* altered with significantly more MU fibers adjacent to one another in reinnervated muscles as compared to the typical mosaic distribution with few adjacent MU muscle fibers, in normally innervated muscles. The more distal nerve branching within the denervated intramuscular nerve sheathes restored the normal size relationships in reinnervated muscles despite the altered branching patterns; and 3) Slow motor nerves demonstrated a preferential reinnervation of denervated endoneurial tubes that had formerly contained these nerves. With thanks to the CIHR for their financial support.

Disclosures: **T. Gordon:** None. **J.E. Totosy de Zepetnek:** Other; jtotosydezepetnek0@shire.com.

Poster

675. Peripheral Nervous System Regeneration

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 675.08/B10

Topic: A.04. Transplantation and Regeneration

Support: CIHR Grant RMF82496

Title: A randomized controlled trial on electrical stimulation to accelerate axon regeneration and functional recovery following cubital tunnel surgery

Authors: *K. CHAN[1], H. A. POWER[2], M. J. MORHART[2], J. L. OLSON[2];
[2]Plastic Surgery, [1]Univ. Alberta, Edmonton, AB, Canada

Abstract: Introduction
Brief post-surgical electrical stimulation (ES) enhances motor and sensory axonal regeneration in animal models following axotomy and crush injury. The ulnar nerve that innervates the majority of hand muscles makes a vital contribution to hand strength. In this study, we investigated the hypothesis that ES following cubital tunnel surgery in patients with severe ulnar nerve injury would result in significantly better muscle reinnervation and functional recovery compared to surgery alone.
Methods
Patients with severe axonal loss from ulnar nerve compression at the elbow were randomly assigned to the treatment or control group in a 2:1 ratio. Those in the control group received cubital tunnel surgery and sham stimulation, while patients in the treatment group received 1 hour of 20Hz ES following surgery. Stimulation was delivered via two stainless electrodes placed adjacent to the ulnar nerve intraoperatively. Patients were followed yearly for 3 years. At each visit, axonal regeneration was quantified using motor unit number estimation (MUNE) and functional recovery was evaluated using grip strength and key pinch strength. Statistical analysis was performed using non-parametric tests, with statistical significance set at $p<0.05$.
Results
Twenty-four patients were enrolled in the study: 8 received surgery alone and 16 received surgery and ES. There was no significant difference in demographics between the two groups. At three years following surgery, MUNE was significantly higher in the treatment group (182±25, mean±sd) compared to controls (93±14, $p<0.05$). In terms of functional recovery, grip strength was significantly improved in the treatment group (43±3kg) at 3 years post-operatively compared to controls (39±3kg, $p<0.05$). Key pinch strength was also significantly better in the treatment group (5.2±0.5kg) compared to controls (4.4±0.8kg, $p<0.05$).
Conclusions
These results suggest that post-surgical ES enhances axonal regeneration, muscle reinnervation

and functional recovery following cubital tunnel surgery in humans. We propose that ES may be a clinically useful adjunct to surgical release for severe ulnar nerve injuries where functional recovery with conventional treatment is poor.

Disclosures: K. Chan: None. **H.A. Power:** None. **M.J. Morhart:** None. **J.L. Olson:** None.

Poster

675. Peripheral Nervous System Regeneration

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 675.09/B11

Topic: A.04. Transplantation and Regeneration

Support: CIHR

UHF

AIHS

Title: Satellite glial cell renewal in dorsal root ganglia after peripheral nerve injury involves self renewal and intrinsic reprogramming of non-glial cells

Authors: *A. KRISHNAN[1], C. CHENG[2], D. W. ZOCHODNE[1];
[1]Dept. of Med., Univ. of Alberta, Edmonton, AB, Canada; [2]Clin. Neurosciences, Univ. of Calgary, Calgary, AB, Canada

Abstract: The generation of new satellite glial cells (SGC) in dorsal root ganglia (DRG) after peripheral nerve injury has previously been described but how they appear is not well understood. In this work we examined proliferative events within rat dorsal root ganglia, three days after sciatic nerve axotomy, to understand overall cellular dynamics. We encountered five main perineuronal cell types relevant to this question: (i) $GFAP^+$ $sox2^+$, (ii) $sox2^+$ $ki67^+$, (iii) $GFAP^+$ $ki67^+$, (iv) $ki67^+$ $GFAP^-$ $sox2^-$, and (v) $sox2^+$ $ki67^-$ $GFAP^-$. Population (i) potentially indicates activation ($GFAP^+$) and dedifferentiation ($sox2^+$) of existing SGCs while, (ii) &(iii) represent proliferation ($ki67^+$) of these de-differentiated ($sox2^+$) SGCs ($GFAP^+$) for self renewal. Interestingly, the population (iv), indicates proliferation of cell types other than SGCs while (v), potentially indicates reprogramming of these unknown cell types. Vimentin positivity in population (iv) & (v) indicate that these cells are of mesenchymal origin. Among all the cell types, population (iv) was seen also in the nerve roots suggesting local proliferation at that site with potential follow up migration of non-glial cells from distant territories to DRGs. We also marked the proliferating cells with Brdu and encountered results supporting the above conclusions. Overall, our observations suggest that SGC generation in response to peripheral

nerve injury involves self renewal of existing SGCs and *in vivo* reprogramming of non-glial mesenchymal cells. The intrinsic reprogramming potential of cell populations in the DRG and nerve roots may shed light on other possibilities such as the potential for generation of new sensory neurons. [Supported by CIHR, UHF, AIHS]

Disclosures: **A. Krishnan:** None. **C. Cheng:** None. **D.W. Zochodne:** None.

Poster

675. Peripheral Nervous System Regeneration

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 675.10/B12

Topic: A.04. Transplantation and Regeneration

Support: NS057190

Title: Repeated application of 20 Hz electrical stimulation after peripheral nerve injury promotes motor axon regeneration and retention of exaggerated H reflexes.

Authors: S. PARK, *A. W. ENGLISH;
Dept Cell Biol., Emory Univ. Sch. of Med., Atlanta, GA

Abstract: A single treatment with one hour of 20 Hz continuous electrical stimulation promotes the regeneration of axons in cut peripheral nerves. This enhanced axon regeneration is the result of a transient increase in neuronal expression of BDNF and its trkB receptor (Al-Majed et al, Eur J Neurosci, 2000. 12:4381-4390). A slightly more robust enhancement of peripheral axon regeneration is achieved by two weeks of daily treadmill exercise, also in a BDNF/trkB-dependent manner. We tested the hypothesis that repeated application of brief electrical stimulation (rES) would be more effective in promoting functional muscle reinnervation than a single application (sES). Sciatic nerves of C57B6 mice were cut and repaired by end-to-end anastomosis. At the time of nerve repair and every third day for the following two weeks, the proximal segment of the cut nerve was stimulated continuously for one hour at 20 Hz. In control groups used for comparison, nerves were cut and repaired and mice were either untreated (UT) or treated with a single application of electrical stimulation (sES) at the time of nerve repair. Beginning two weeks later, functional muscle fiber reinnervation was assayed using stimulus evoked EMG activity from the gastrocnemius and tibialis anterior muscles. Direct muscle (M) responses and monosynaptic H reflexes produced in response to sciatic nerve stimulation above the injury site were studied in awake animals. The amplitude of M responses recorded from reinnervated muscles increased progressively over the 14 week post transection study period. In the rES animals, this increase was more rapid than sES or UT mice. In rES mice, the amplitude

of H reflexes recorded from reinnervated muscles increased more rapidly than found in either UT or sES mice, reaching a peak at six weeks after nerve injury. The H reflexes in the rES animals were maintained at more than twice the amplitude of the same reflexes recorded prior to injury for the remaining eight weeks studied. Repeated ES does enhance motor axon regeneration and functional muscle reinnervation, and this enhancement is more robust than a single ES treatment, but rES also results in the retention of exaggerated H reflexes.

Disclosures: S. Park: None. **A.W. English:** None.

Poster

675. Peripheral Nervous System Regeneration

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 675.11/B13

Topic: A.04. Transplantation and Regeneration

Support: CIHR

CDA

Title: A growth suppressive role for major urinary protein (MUP) expression in diabetic and nondiabetic adult sensory neurons

Authors: *D. W. ZOCHODNE[1,2], V. SINGH[3], A. CHANDRASEKHAR[1,2], T. POITRAS[2], J. A. MARTINEZ[3];
[1]Med. and Neurol., [2]Univ. of Alberta, Edmonton, AB, Canada; [3]Univ. of Calgary, Calgary, AB, Canada

Abstract: In an array analysis of gene expression in dorsal root ganglia (DRGs) of mice we encountered an unexpected upregulation of major urinary proteins (MUPs 1,2) in chronic experimental diabetes. MUPs were initially identified as secretory proteins in urine with a potential role as pheromones, among other possibilities. Mice with chronic experimental diabetes provide a model that exhibits key features of human diabetic polyneuropathy with electrophysiological abnormalities (motor and sensory conduction slowing) and behavioural changes (loss of thermal sensation). Here, we confirmed the prominent and widespread cytosolic expression of MUPs in both mouse and rat sensory neurons and in DRG glial cells. Moreover, MUPs were expressed in sciatic axons, Schwann cells and distal sensory branches within the skin dermis of the footpad. In a chronic diabetes model, knockdown of MUP2 through intranasal nonviral siRNA delivery had an impact on downstream axonal function, improving motor and sensory conduction abnormalities. Anticipating that MUP may have a functional impact on

overall growth plasticity of sensory neurons, we also examined the impact of its knockdown on neurite outgrowth of dissociated and preinjured rat DRG sensory neurons. MUP1,2 knockdown was associated with a significant rise in neurite outgrowth compared to neurons exposed to control scrambled siRNA sequences. Moreover, this impact was confirmed separately in adult mouse neurons. Taken together, our findings suggest unique and functional roles for MUPs in sensory axons that may differ from their potential pheromone actions. Overall, MUPs appear to restrain growth in adult neurons. Their upregulation in experimental diabetes may contribute, along with other abnormalities of sensory gene expression, to the complex phenotype of this disorder. [Supported by CIHR, CDA]

Disclosures: D.W. Zochodne: None. **V. Singh:** None. **A. Chandrasekhar:** None. **T. Poitras:** None. **J.A. Martinez:** None.

Poster

675. Peripheral Nervous System Regeneration

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 675.12/B14

Topic: A.04. Transplantation and Regeneration

Support: Alberta Innovates-Health Solutions Sustainability Funding Program and University of Calgary Cumming School of Medicine Bridge Funding Program

University Research Grants Committee Seed Grant

Alberta Innovates-Health Solutions Doctoral Studentship

Title: AlphaB-crystallin modulates immune responses and lipid metabolism in an age dependent matter in the uninjured and damaged peripheral nervous system.

Authors: E.-M. LIM[1], *S. S. OUSMAN[2];
[1]Neurosci., [2]Clin. Neurosciences and Cell Biol. & Anat., Hotchkiss Brain Institute, Univ. of Calgary, Calgary, AB, Canada

Abstract: As the peripheral nervous system (PNS) ages, immune responses and oxidative stress are enhanced while lipid metabolism is reduced. Further, Wallerian degeneration is delayed in an age dependent manner following PNS injury. To determine what underlies the aging-related deficits in the undamaged and injured PNS, we noted that a small heat shock protein called alphaB-crystallin (αBC), which is expressed by PNS axons and Schwann cells, is significantly reduced with age. To evaluate whether αBC contributes to PNS aging related processes, we assessed for presence of macrophages (Iba1 staining), and for markers of lipid metabolism

(SQLE staining) and oxidative stress (CHOP immunohistochemistry). We found that naïve and 28d injured αBC null mice exhibited increased Iba1+ profiles at 3 and 12 months of age compared to WT counterparts. This augmented immune response correlated with enhanced levels of chemokines at particular time points after injury in the knockout animals. With respect to lipid metabolism and oxidative stress, the number of SQLE profiles was markedly decreased in 12 month old null mice while the counts for CHOP remained unchanged. Altogether, these data demonstrate that the reduced levels of αBC in sciatic nerves with age correlated with a deficit in lipid metabolism and, an exuberant macrophage presence.

Disclosures: E. Lim: None. **S.S. Ousman:** None.

Poster

675. Peripheral Nervous System Regeneration

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 675.13/B15

Topic: A.04. Transplantation and Regeneration

Title: Chronic electrical muscle stimulation (EMS) following nerve injury and repair in Sprague Dawley rats does not lead to preferential reinnervation of target muscles

Authors: *M. WILLAND[1], J. CATAPANO[1], J. BURGUET[2], P. ANDREY[2], G. H. BORSCHEL[1], T. GORDON[1];
[1]Div. of Plastic Reconstructive Surgery, The Hosp. For Sick Children, Toronto, ON, Canada;
[2]Inst. Jean-Pierre Bourgin, Versailles, France

Abstract: Regenerating motor nerves randomly reinnervate target muscles resulting in synkinesis. The efficacy of chronic EMS on nerve regeneration is controversial. In a canine model of laryngeal nerve injury, EMS of reinnervating muscles promotes selective and appropriate reinnervation of muscles. This evidence is largely based on functional measurements. We provide direct evidence through retrograde labeling and spatial analysis of neuronal populations that EMS does not preferentially reinnervate target muscles.
The soleus and lateral gastrocnemius (LG) muscles in two groups of rats were used as the target muscles. Soleus muscles were injected with True Blue (TB) fluorescent tracer to label the original soleus motoneuron pool. One week later, bipolar stainless steel wire electrodes were implanted into the soleus muscle. The lateral gastrocnemius soleus (LGS) nerve was then transected and immediately repaired using one epineurial suture (11-0). The experimental group of rats underwent EMS of the soleus muscle with 12 hrs of EMS per day at 20 Hz (10 sec on, 20 sec off, 400 μs pulse width) followed by 12 hrs of intermittent stimulation (10 sec on, 1 hr off) performed 7 days per week. Two months following nerve injury and repair the soleus nerve was

retrogradely labelled with Fluoro-Ruby (FR). The contralateral uninjured side was also labelled to assess the distribution of normal motoneuron pools. Labeled motoneurons were counted, and where possible, spatially normalized models were created to analyze and compare the spatial distributions between groups. In a subset of rats in which the motoneuron pools could not be spatially normalized, the nearest-neighbour distance distribution function was used for analysis. The number of motoneurons that labeled with both TB and FR were not different whether or not the soleus muscle was stimulated demonstrating that EMS did not promote preferential reinnervation of the muscle. However, retrograde labeling via intramuscular injection only labeled ~75% of the soleus motoneurons. Spatial statistics were used to provide a more detailed examination of the segregation of the soleus and LG motoneuron pools. The pools show a clear segregation in the dorsal-ventral plane in uninjured rats. However, nearest-neighbour analysis demonstrated that the reinnervated motoneuron pools were highly segregated as compared to the original soleus motoneuron pools *and* the EMS did not affect the degree of segregation.
Our findings here demonstrate that chronic EMS of reinnervating muscle does not promote preferential reinnervation of target muscles but, importantly, it does not prevent muscles from being reinnervated by regenerating nerves.

Disclosures: M. Willand: None. **J. Catapano:** None. **J. Burguet:** None. **P. Andrey:** None. **G.H. Borschel:** None. **T. Gordon:** None.

Poster

675. Peripheral Nervous System Regeneration

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Topic: A.04. Transplantation and Regeneration

Support: Saskatchewan Health Research Foundation (2803)

Canadian Institutes of Health Research (MOP74747, 142328)

Title: Expression of the monocyte chemokine CCL2 in regenerating peripheral nerve is rapidly elevated in response to brief electrical nerve stimulation

Authors: J. M. JOHNSTON, R. ZHAI, N. A. MCLEAN, J. R. NADEAU, *V. M. VERGE; Anat. & Cell Biol., Univ. Saskatchewan-CMSNRC, Saskatoon, SK, Canada

Abstract: The neural immune axis plays a critical role in peripheral nerve regeneration, with previous studies linking overexpression of CCL2/MCP-1 to an enhanced regenerative capacity akin to a conditioning lesion (Kwon et al., 2015 J Neurosci 35(48):15934; Niemi et al, 2016 Exp

Neurol 275(1):25-37). We and others have shown that electrical nerve stimulation (ES) is an effective adjunct therapy to enhance nerve regeneration in manner akin to a conditioning lesion (reviewed in Gordon and Borschel 2016 Exp Neurol). What is not known is whether this is linked to an impact on the neural immune axis with respect to regulation of CCL2 expression and monocyte recruitment. To examine this, we conducted a time course analysis on adult male Wistar rats subjected to a unilateral mid thigh sciatic nerve crush with 1 hour continuous electrical nerve stimulation at 20 Hz at the time of nerve injury. Injury only and injury + ES with lidocaine block served as controls. Preliminary data reveals a rapid response to ES with respect to CCL2 expression as early as 3 hours post-ES, when a discernible increase in CCL2 expression was evident in the Schwann cells of the regenerating nerve proximal to injury with a more robust increase observed at the level of the dorsal root ganglion (DRG) in sensory neurons and axons. By 2 days post-injury the nerves subjected to ES had even higher levels of CCL2 expression relative to the non-stimulated nerves in both axons and Schwann cells, the former suggesting anterograde transport of CCL2 in the sensory axons to the regenerating front. Further, there was also a corresponding increase in the numbers of macrophages observed in the injury zone of the stimulated nerve. As with the 3 hr timepoint, the 2 day ES-associated increases in CCL2 expression was even higher in the corresponding DRG neurons and axons. Analysis of the additional timepoints will reveal how long this response is sustained. Collectively, these results demonstrate an impact of ES on an aspect of the neural immune response known to benefit axon regeneration.
This research is supported by Saskatchewan Health Research Foundation (2803) and Canadian Institutes of Health Research (MOP74747, 142328) grants to VMKV.

Disclosures: **J.M. Johnston:** None. **R. Zhai:** None. **N.A. McLean:** None. **J.R. Nadeau:** None. **V.M. Verge:** None.

Poster

675. Peripheral Nervous System Regeneration

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Topic: A.04. Transplantation and Regeneration

Support: DK097223

EY11373

2R25GM075207

Title: Global knockout of Toll-like Receptors 2 and 4 does not hinder Wallerian degeneration but does somewhat impair regeneration after sciatic nerve injury

Authors: *J. LINDBORG, O. SHELTON, R. ZIGMOND;
Neurosciences, Case Western Reserve Univ., Cleveland, OH

Abstract: Peripheral neurons can regenerate after a nerve injury. The axon distal to the site of injury degenerates and the debris is cleared by macrophages (mφs) and Schwann cells (a process called Wallerian degeneration), which allows regenerating axons to extend from the proximal nerve segment. Mφs accumulate after nerve injury in the distal nerve segment and near the cell bodies. Although it has been shown that without mφ accumulation there is hindered degeneration and regeneration, the mechanisms by which mφs are able to accumulate and aid in these processes are incompletely understood. It has been proposed that Schwann cells, neurons and satellite glial cells induce the release of the chemokine CCL2 after nerve injury via the activation of the Toll-like receptors (TLRs) 2 and 4, which results in the accumulation of mφs at the cell body and distal to the site of injury. We have investigated the importance of these receptors in mφ accumulation, myelin clearance and regeneration in dorsal root ganglia (DRG), superior cervical ganglia (SCG), and sciatic nerves after injury in wildtype (WT) and TLR 2/4 double knockout (DKO) mice. Our results show variable accumulation of mφs in all tissues depending on the specific marker used. $CD68^+$ mφ accumulation is unchanged between genotypes in all tissues 7d following axotomy. However, accumulation is decreased in all tissues in DKO mice when using CD11b to label mφs. $Iba1^+$ mφ accumulation is comparable between genotypes in the sciatic nerve, but is significantly decreased in DKO DRG compared to WT DRG. At present, we do not know the significance of the differential expression of mφ markers. While myelin clearance is comparable between genotypes 7d after axotomy, L5 DRG explants of DKO mice show a small but significant decrease in neurite outgrowth relative to WT DRG after 48h in culture. Interestingly, the loss of TLRs 2 and 4 has no effect on CCL2 mRNA expression at 24 or 48h after injury in all three tissues of DKO mice, except in the sciatic nerve at 24h post-injury where we observe a significant decrease compared to WT mice. In the DRG and SCG 24h after axotomy, we observed no differences between genotypes in mRNA expression of Csf1, GFAP, c-Jun, or CX3CL1. c-Jun expression at 24h after injury was significantly increased in WT sciatic nerve compared to DKO mice, although no difference was observed at 48h. c-Jun expression at 48h post-injury was significantly increased in DKO DRG compared to WT DRG, but the converse was found in SCG. In conclusion, TLR 2/4 signaling has no effect on myelin clearance after injury, but exerts a small yet significant effect on nerve regeneration. (Supported by DK097223, EY11373, and 2R25GM075207)

Disclosures: J. Lindborg: None. **O. Shelton:** None. **R. Zigmond:** None.

Poster

675. Peripheral Nervous System Regeneration

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Topic: A.04. Transplantation and Regeneration

Support: DK097223

NS077888

EY11373

Title: Classification of macrophage activation state in dorsal root ganglia after peripheral nerve injury

Authors: M. HOWARTH[1], *A. DEFRANCESCO-LISOWITZ[2], J. P. NIEMI[3], C. MOORE[4], R. E. ZIGMOND[3];
[1]Hathaway Brown Sch., Shaker Heights, OH; [2]Neurosci., [3]Neurosciences, [4]Med. Physiol., Case Western Reserve Univ., Cleveland, OH

Abstract: Understanding the complex process by which peripheral neurons regenerate plays an integral role in the ability to develop therapies for injury both to the peripheral nervous system and to the normally non-regenerating central nervous system. Previous research conducted by our laboratory suggested that the regenerative process is dependent on post-axotomy macrophage accumulation not only in the segment of the nerve distal to the site of injury, as previously established, but also around the neuronal cell bodies, located in peripheral ganglia. However, the exact method by which these macrophages assist in regeneration is unknown due to a poor understanding of their properties, including whether they behave in a pro- or anti-inflammatory manner. Thus, to obtain a better understanding of the function of these macrophages, we performed a phenotypic analysis of macrophages in the lumbar level 5 dorsal root ganglion (DRG) after sciatic nerve transection. Real-time PCR analysis was used to determine the time course of pro- and anti-inflammatory macrophage markers in DRG after injury. Interestingly, a significant increase in the anti-inflammatory markers CD206, Ym1, and Arginase 1 occurred only at 7 days after injury compared to the early upregulation of the pro-inflammatory markers CD86 and iNOS occurring at 3 days post-injury. These data raise the possibility that macrophages are not polarized to an anti-inflammatory activation state when they first accumulate in the DRG but rather shift their activation over time. To specifically localize these markers to macrophages, immunohistochemistry was performed to label macrophages, using antibodies to CD11b and CD68, and antibodies to markers expressed by anti-inflammatory macrophages, using CD206, and pro-inflammatory macrophages, using iNOS, in the DRG at various times after injury. Our analysis suggests that macrophages in the DRG 7 days after

injury, but not at earlier time points, highly colocalize with the anti-inflammatory phenotypic marker, CD206. Staining was also carried out in the CCR2 knockout mouse, a mouse that has a significantly diminished macrophage response to injury. CCR2 knockout mice displayed a significant reduction in CD206 expression, as a result of the reduced macrophage population within the ganglia. Taken together, these data suggest that macrophages accumulating in axotomized peripheral ganglion tend towards an anti-inflammatory phenotype at 7 days. (Supported by DK097223, NS077888, and EY11373)

Disclosures: **M. Howarth:** None. **A. Defrancesco-Lisowitz:** None. **J.P. Niemi:** None. **C. Moore:** None. **R.E. Zigmond:** None.

Poster

675. Peripheral Nervous System Regeneration

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Topic: A.04. Transplantation and Regeneration

Support: DK097223

NS077888

EY11373

Title: Macrophage stimulation of peripheral axon regeneration requires CCL2 and pSTAT3

Authors: ***J. P. NIEMI**[1], A. DEFRANCESCO[1], C. MOORE[2], R. E. ZIGMOND[1]; [1]Neurosciences, [2]Med. Physiol., Case Western Reserve Univ., Cleveland, OH

Abstract: Neuroinflammation plays a critical role in the regeneration of peripheral nerves following axotomy. An injury to the sciatic nerve leads to significant macrophage accumulation in the L5 DRG, an effect not seen when the dorsal root is injured. Recent evidence showed that macrophage accumulation around axotomized cell bodies is necessary for a peripheral conditioning lesion response. In response to an axonal injury, DRG neurons upregulate and release CCL2, a macrophage chemokine which acts on the receptor CCR2. In a CCR2 knockout mouse (CCR2-/-), CD11b+ macrophage accumulation was inhibited in the distal sciatic nerve and in the axotomized DRG after injury. Increased outgrowth was seen in previously lesioned DRGs from wild type but not CCR2-/- mice. These data suggest a relationship between macrophage accumulation near neuronal cell bodies and the regenerative capacity of neurons as well as highlighting the role CCL2/CCR2 signaling plays in mediating macrophage entry into DRGs. We asked whether overexpression of CCL2 specifically by DRG neurons of uninjured

mice is sufficient to cause macrophage entry and enhanced regeneration, or whether other injury-derived signals are necessary. We found that CCL2 could be significantly overexpressed in DRG neurons by utilizing an adeno-associated virus (AAV) encoding for CCL2 driven by the EF1α promoter injected intrathecally. The injection led to a time dependent increase in CCL2 expression and in macrophage accumulation in the L5 DRG compared to controls injected with virus expressing YFP. CCL2 overexpression and subsequent macrophage accumulation led to a conditioning-like increase in neurite outgrowth of DRG neurons in explant and dissociated culture. An AAV-CCL2 injection resulted in increased LIF mRNA in the DRG and increased neuronal phospho-STAT3. Blockade of STAT3 activation, through the administration of STAT3 phosphorylation inhibitors, AG490 or Stattic, reduced or completely ablated, respectively, the CCL2 overexpression-induced increase in axonal outgrowth. To demonstrate the necessity of macrophage accumulation in stimulating the increased axonal regeneration observed after CCL2 overexpression, a CSF-1 receptor inhibitor will be employed to prevent macrophage accumulation in the DRG. Further studies into the necessity of neuronal expression of CCL2 after injury are also being carried out using a DRG sensory neuron specific knockout of CCL2. Together, these data indicate that neuronal CCL2 expression and macrophage accumulation within the DRG are both necessary and sufficient for peripheral axonal regeneration to occur. (Supported by DK097223, NS077888, and EY11373)

Disclosures: **J.P. Niemi:** None. **A. DeFrancesco:** None. **C. Moore:** None. **R.E. Zigmond:** None.

Poster

675. Peripheral Nervous System Regeneration

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Topic: A.04. Transplantation and Regeneration

Support: DK097223

K18 DC013304

Title: Oncomodulin is not essential for a conditioning lesion effect in mouse dorsal root ganglia

Authors: A. DEFRANCESCO[1], A. J. HORNAK[2], D. D. SIMMONS[2], *R. E. ZIGMOND[3]; [1]Neurosciences, Case Western Reserve Univ., Cleveland, OH; [2]Integrative Biol. and Physiol., UCLA, Los Angeles, CA; [3]Case Western Res. Univ. Sch. Med., Cleveland, OH

Abstract: A conditioning lesion (CL) of the sciatic nerve increases neurite outgrowth measured subsequently in explant and dissociated cultures of L4 and L5 dorsal root ganglia (DRGs), effects that are dependent on macrophage accumulation in these DRGs (for review see DeFrancesco-Lisowitz et al., Neuroscience 302:174, 2015). The molecule secreted by macrophages that is responsible for triggering the CL effect has not been identified. Oncomodulin has been proposed to account for the beneficial effect of neuroinflammation on axonal growth by retinal ganglion cells and to be secreted by macrophages and neutrophils (for review see Benowitz et al., Exp Neurol 2016 in press). Oncomodulin is also expressed in DRGs after axotomy and is required for the increased outgrowth in cultured neurons produced by conditioned medium from macrophage-neuron co-cultures treated with dibutyryl-cAMP (Kwon et al., J Neurosci 33:15095, 2013). Since it is unknown whether oncomodulin plays a role in the CL effect, we examined this effect in oncomodulin knockout (KO) and wild type (WT) mice (Tong et al., J Neurosci 36: 1631, 2016). Seven days after unilateral sciatic nerve transection, L4 and L5 DRGs were removed. L4 ganglia were dissociated and cultured for 24 h in the absence of added growth factors. The neurons were then stained for βIII tubulin and outgrowth determined. The length of the longest neurite per neuron was measured as well as the total neurite outgrowth. For both measures, a CL increased outgrowth in both KO and WT ganglia, though the effect was somewhat smaller in the KO mice. The ratio of the mean length of the longest neurite in neurons from axotomized DRGs to that in neurons from sham-operated ganglia was 3.2 for WT tissues and 2.5 for KO tissues ($p<0.01$; all statistics reflect differences between genotypes). The ratio of the total neurite outgrowth in axotomized compared to sham-operated neurons was 4.1 for WT ganglia and 3.3 for KO ganglia ($p<0.05$). L5 ganglia were placed in explant cultures in Matrigel, and neurite outgrowth was measured at 24 and 48 h under phase microscopy. Again, a CL effect was seen in DRGs from both genotypes, but the effect was somewhat smaller in the KO DRGs (mean length of 20 longest neurites at 48 h: WT=0.93 mm and KO=0.72 mm; $p<0.01$). Since macrophages also play an important role in Wallerian degeneration, we asked whether oncomodulin is involved in myelin clearance but found no significant difference in clearance in the distal sciatic nerve between genotypes. In conclusion, our results indicate that oncomodulin plays only a small, though significant, role in the CL effect in DRGs. [Supported by grants DK097223 (REZ) and K18 DC013304 (DDS)]

Disclosures: A. DeFrancesco: None. **A.J. Hornak:** None. **D.D. Simmons:** None. **R.E. Zigmond:** None.

Poster

675. Peripheral Nervous System Regeneration

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Topic: A.04. Transplantation and Regeneration

Support: JSPS KAKENHI 15H05041

Title: Axonal regeneration requires activation of Sonic hedgehog signaling pathway in peripheral nerve injury

Authors: *Y. YAMADA[1], T. MAEDA[2], A. OHAZAMA[3], K. SEO[1];
[1]Dent. Anesthesiol., Grad. Sch. of Niigata Univ., Niigata, Japan; [2]Ctr. for Advanced Oral Sci., [3]Oral anatomy, Niigata Univ. Grad. Sch. of Med. and, Niigata, Japan

Abstract: [Introduction] When the peripheral nerve is transected completely, their transected nerve ends can meet together to reconnection. However, its mechanism remains unclear. Sonic hedgehog (Shh) signaling pathway plays critical roles in axonal guidance (Charron et al. Cell 2003) in addition to determining the proximal and distal direction during organogenesis. Our previous report has demonstrated the peripheral nerve injury induces the expression of Shh and its receptor (Patched1) in the distal and proximal neve ends of , respectively (Annual meeting Sfn 2015), suggesting a possible involvement of Shh signaling in nerve regeneration. Aim This study aims to clarify the expression pattern of Shh signaling pathway during neural regeneration in a nerve injury model. [Materials and methods] Male C57BL6 mice (7-8 weeks old) were used in this study. Transgenic mice expressing GFP under the control of Gli1 (the transcription factor) promoter (Gli1-GFP) were also used to identify which cell type induces Gli1 expression after nerve injury. Under an anesthesia, the inferior alveolar nerve (IAN) was exposed and completely transected in both kinds of mice. They were sacrificed at 1, 3, 7 and 14 days post-surgery. In some mice, cyclopamine, an inhibitor of Shh signaling pathway was local administrated at every 24 hours for 14 days. This study examined 1) the chronological changes in expression pattern of Shh and Patched1 during regeneration of injured IAN by in situ hybridization, 2) compared Gli1 expression with neuronal and non-neuronal markers molecules by immunohistochemistry, and 3) assessed the nerve regeneration process in mice with cyclopamine by immunohistochemistry for PGP9.5. [Results] 1) The distal stamp of transected IAN showed the expressions of Shh in contrast to Patched1 expression at proximal stump at 1day after surgery. This expression pattern reduced at 7days after surgery. 2) We found a co-localization of Gli1-GFP and p75-reactions. 3) Sprouting axons neuron from distal and proximal stumps were found in random directions in cyclopamine group. [Conclusion] The Shh signaling pathway is involved in reconnection of transected axonal ends and regrowth of axon during the early stages of regeneration.

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Poster

675. Peripheral Nervous System Regeneration

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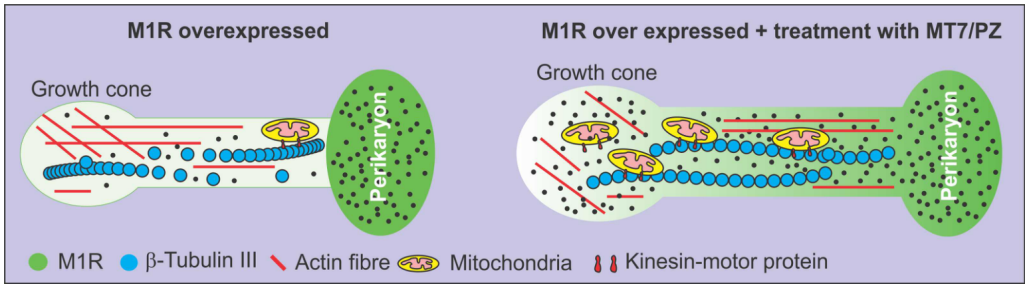
Topic: A.04. Transplantation and Regeneration

Support: CIHR grant # MOP-130282

Title: Over-expression of muscarinic acetylcholine type 1 receptor causes cytoskeletal abnormalities and impairs mitochondrial trafficking in adult sensory neurons.

Authors: *M. G. SABBIR[1], P. FERNYHOUGH[2,3];
[1]St. Boniface Res. Ctr., Winnipeg, MB, Canada; [2]1Division of Neurodegenerative Disorders, St. Boniface Hosp. Res. Ctr., Winnipeg, MB, Canada; [3]Dept of Pharmacol. & Therapeut., Univ. of Manitoba, Winnipeg, MB, Canada

Abstract: Muscarinic acetylcholine receptors are a subfamily of G protein-coupled receptor that regulate numerous fundamental biological pathways in the central and peripheral nervous systems. It has been shown in a non-mammalian system that neurotransmitters such as acetylcholine can direct axonal growth during development. Modulation of these cholinergic pathways could be therapeutically useful for neurodegenerative diseases such as peripheral neuropathy in which sensory nerve terminals are gradually depleted. Recently, we have shown that selective or specific muscarinic acetylcholine type 1 receptor (M1R) antagonists can induce a dose-dependent elevation in neurite outgrowth. The exact mechanism of M1R-antagonist driven neurite outgrowth is not understood. In order to understand the biological function of M1R in peripheral neurons, we have overexpressed M1R in primary adult dorsal root ganglion (DRG) sensory neurons and studied the physiological as well as molecular effects. Interestingly, we have found M1R overexpression caused significant ($p<0.005$) depletion of functional mitochondria at the growth cone and a subsequent decrease in neurite outgrowth ($p<0.0001$). The diminished abundance of mitochondria in axons was associated with discontinuity in the β-tubulin cytoskeleton structure that, in turn, suppressed mitochondrial trafficking. The tubulin associated cytoskeletal defect was corrected by treatment with muscarinic antagonists pirenzepine (PZ) and muscarininc toxin 7 (MT7) which mediated recruitment of G proteins and increased Ca^{2+} signaling in the neurites. In accordance with this finding, we observed significant increase in neurite outgrowth in rat sensory neurons when treated with 100nM MT7 or 1μM PZ, respectively. Our findings suggest a novel mechanism in which modulation of M1R influences mitochondrial distribution in nerve terminals and controls axonal growth and regeneration.



Disclosures: **M.G. Sabbir:** A. Employment/Salary (full or part-time): Albrechtsen Research Centre, St. Boniface Hospital. **P. Fernyhough:** None.

Poster

675. Peripheral Nervous System Regeneration

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DOD Grant W91ZSQ2136N601

Title: Electrospun polymer-polyvalent hydrogel composite fiber scaffolds with encapsulated neurons and glia for neural repair

Authors: ***J. M. COREY**[1,2], C. CHAN[3,2], C. M. WHITE[3,2], A. RASTOGI[3,2], A. M. GRANT[3,2], R. J. MILLER[3], R. K. DUNCAN[3];
[1]Dept Neurol, Univ. of Michigan Dept. of Neurol., Ann Arbor, MI; [2]VA Ann Arbor Healthcare Syst., Ann Arbor, MI; [3]The Univ. of Michigan, Ann Arbor, MI

Abstract: Aligned polymer nanofibers made by electrospinning provide powerful topographical guidance cues in nerve guidance channels and other tissue engineering applications. Seeding these biomimetic scaffolds with transplanted Schwann cells or stem cells seems straightforward, but small pores inherent in electrospun fiber scaffolds limit depth of cell penetration to only 1-2 cell diameters. One potential solution is to construct electrospun fibers scaffolds to include polyvalent hydrogel (PVH) fibers in which cells can be encapsulated so that cells can be released and contact the polymer fibers for topographic guidance. While recent work has shown that endothelial and other cell types can be encapsulated by PVH, cells from the nervous system can

be much more sensitive to insult in culture and artificial biomaterials. Therefore we sought to test the suitability of PVH microfibers with cells from the nervous system. We hypothesized that primary neurons would have a more difficult time surviving in PVH than fibroblasts or glia. Aligned poly-L-lactide nanofibers were electrospun on to substrates. L929 fibroblasts, primary astrocytes, or primary cortical neurons were mixed into a 2.5% alginate solution. PVH fibers were constructed by justaposing a large drop of this solution with 2% chitosan in 0.2M acetic acid and manually pulling the interface. The PVH fiber was guided on nanofiber substrates attached to a rotating mandrel. Substrates were cultured for 30 m, 4 h, 1 d, and 7 d, prior to staining with a Calcein-AM viability assay. We found that all cell types survived well outside PVH fibers, ranging from 86±0.8% for cortical neurons to 97±0.02% for L929 fibroblasts. In PVH fibers, survival of L929 fibroblasts was lowest at 30 m (61±17%) and steadily increased to a high of 88±05% at 1 d, and falling slightly to 87±12% at 7 d. Astrocytes viability was somewhat less at 47±8% at 30 m but climbing steadily to 74±10% at 7 d. The increase in cell number over the 7 d time window of both astrocytes and L929 cells suggests that cell proliferation may be occurring inside the hydrogels. Cortical neuron viability was worse, ranging from 12±13% at 30 m to a low of 6.3±4% at 4h. These data are consistent with our hypothesis that primary neurons, being a very sensitive cell type, would fare poorest. These experiments may be the first to encapsulate cells from the CNS into PVH fibers. To encapsulate primary neurons at useful survival percentages, PVH fibers will likely require modification by adjusting pH or addition of growth factors. Further work will center on studying the release of cells from the PVH fibers and attraction to and interaction with the electrospun nanofibers.

Disclosures: **J.M. Corey:** None. **C. Chan:** None. **C.M. White:** None. **A. Rastogi:** None. **A.M. Grant:** None. **R.J. Miller:** None. **R.K. Duncan:** None.

Poster

675. Peripheral Nervous System Regeneration

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 675.22/B24

Topic: A.04. Transplantation and Regeneration

Support: DOD Award W81XWH-14-1-0442

Title: Outcomes of muscle reinnervation with direct nerve implantation into the native motor zone of the target muscle

Authors: ***S. SOBOTKA**[1,2], J. CHEN[1], T. NYIRENDA[1], L. MU[1];
[1]Dept. of Res., Hackensack Univ. Med. Ctr., Hackensack, NJ; [2]Dept. of Neurosurg., Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: Our recent work has demonstrated that the native motor zone (NMZ) within a given skeletal muscle is the best site for muscle reinnervation. This study was designed to investigate the efficacy of direct nerve implantation (DNI) into the NMZ of denervated sternomastoid (SM) muscle in a rat model. The right SM muscle was experimentally denervated by transecting its innervating nerve at its entrance to the muscle (motor point). The proximal stump of the severed SM nerve was immediately buried into a small muscle slit made in the NMZ of the target muscle. The implanted nerve was then secured with an epineurial suture of 10-0. At the end of the 3-month recovery period, all experimental animals underwent postoperative evaluations to assess surgical outcomes of the DNI-NMZ procedure. Functional recovery and muscle reinnervation were evaluated using electrophysiological and histochemical techniques, respectively. Maximum tetanic force was measured from the treated and contralateral control SM muscles in each animal. The degree of functional recovery was determined by comparing the muscle force of the reinnervated SM muscle with that of the contralateral control muscle. Averaged maximal muscle force at the operated side was 0.763 N, whereas 1.200 N at the contralateral control side. The DNI-NMZ reinnervated SM muscles produced 63.6% of the maximal tetanic force of the control muscles. The difference was statistically significant ($p = 0.0013$). Muscle analyses showed that the treated SM muscle recovered morphologically and histologically as indicated by a good preservation of muscle mass and abundant regenerated axons. Specifically, DNI-NMZ reinnervated SM muscles weighed 71% of the weight of contralateral control muscles. The treated muscles exhibited slight-to-moderate fiber atrophy as compared with the controls. Quantitative analysis showed that the mean number and area of the regenerated axons in the treated muscles was 62% and 51% of the contralateral controls, respectively. The findings from this study suggest that DNI-NMZ holds promise in the treatment of muscle paralysis. For optimal outcome, further studies are needed to promote the efficacy of this technique by further refining surgical procedure and using additional approaches to accelerate axonal regeneration.

Disclosures: **S. Sobotka:** None. **J. Chen:** None. **T. Nyirenda:** None. **L. Mu:** None.

Poster

675. Peripheral Nervous System Regeneration

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 675.23/B25

Topic: A.04. Transplantation and Regeneration

Support: MEXT/JSPS KAKENHI Grant Number 24111515

Title: Phosphoproteomics identifies a phosphorylation site of GAP-43 in the peripheral nerve regeneration

Authors: *M. OKADA[1,2,3], A. KAWASAKI[2], Y. YOSHIDA[4], Y. FUJI[3], M. IGARASHI[1];
[1]Dept. of Neurochemistry and Mol. Cell Biol., [2]Ctr. for Transdisciplinary Res., Niigata Univ., Niigata, Japan; [3]Dept. of Neurosurg., Brain Res. Inst., Niigata, Japan; [4]Dept. of Structural Pathology, Inst. of Nephrology, Niigata university, Niigata, Japan

Abstract: Growth-associated protein 43 (GAP-43) is believed as a classical molecular marker for axon growth/regeneration, since this protein is known to be high expressed and intensively undergoes anterograde transport in the regenerating axon after injury. In our previous study, phosphoproteomic analysis using neonatal rat brains revealed several novel phosphorylation sites of GAP-43. We then successfully generated specific antibody (pGAP-43 Ab) against one of those sites (Kawasaki, Okada, Igarashi *et al.*, submitted).
To test whether this phosphorylation is induced in the axon regeneration, we tried both the immunohistochemistry and the phosphoproteomics using the sciatic nerve injury of adult C57B6N mice (9-weeks-old or later). The crushed nerves (day 3 after injury) and the uninjured control nerves were assessed by western blotting and immunohistochemistry using pGAP-43 Ab. For quantitative evaluation of regeneration, we used the confocal micrographs along the longitudinal nerve section and calculated "Regeneration Index", which is defined by the distance between the crush site (point A) and the site at which the intensity level of pGAP-43 Ab is half of that at point A (*Shin J.E. et al. Neuron 74(2012)*). Immunohistochemistry revealed the very low staining of pGAP-43 Ab in the control nerve. Regeneration Index with the pGAP-43 Ab labeling could be used to measure the extent of Axon regeneration. The signal intensity of bands of pGAP-43 Ab on western blotting was detected higher in the the crushed nerve than that in the control nerve. For mass spectrometric analysis, the protein extracts prepared from the defined portions of either single crushed or uninjured sciatic nerves, underwent phosphoproteomics directly by LC-MS/MS. As results, we succeeded in unambiguously detecting GAP-43-derived peptides containing this phosphorylated site exclusively in the regenerating axon. Taken together, we concluded that this site is the major phosphorylation residue of GAP-43 in the regenerating axon, and the pGAP-43 Ab which quantitatively detected the phosphorylation of GAP-43 could be a novel probe for the axonal regeneration *in vivo*.

Disclosures: M. Okada: None. **A. Kawasaki:** None. **Y. Yoshida:** None. **Y. Fuji:** None. **M. Igarashi:** None.

Poster

675. Peripheral Nervous System Regeneration

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 675.24/B26

Topic: A.04. Transplantation and Regeneration

Support: DOD Award W81XWH-14-1-0442

Title: Muscle reinnervation: modified nerve-muscle-endplate band grafting technique

Authors: *L. MU[1], S. SOBOTKA[1,2], J. CHEN[1], T. NYIRENDA[1];
[1]Dept. of Res., Hackensack Univ. Med. Ctr., Hackensack, NJ; [2]Dept. of Neurosurg., Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: Neural regeneration and functional recovery of the paralyzed skeletal muscle caused by peripheral nerve injuries are major challenges in rehabilitation medicine. This study was designed to modify our recently developed nerve-muscle-endplate band grafting (NMEG) for promoting the efficacy of this muscle reinnervation technique in a rat model. The right sternomastoid (SM) muscle was denervated by resecting a 5-mm segment of its innervating nerve and immediately reinnervated with NMEG. Specifically, a NMEG pedicle was harvested from the native motor zone (NMZ) of the right sternohyoid (SH) muscle and implanted into the NMZ of the ipsilateral experimentally denervated SM muscle. A NMEG contained a muscle block (6 x 6 x 3 mm), a nerve branch with nerve terminals, and a motor endplate band with numerous neuromuscular junctions. A muscular defect (recipient bed) with the same dimensions as the NMEG pedicle was made in the NMZ of the right denervated SM muscle. The well-prepared NMEG was embedded in the SM muscle defect and sutured with four to six 10-0 nylon microsutures. Three months after surgery, maximum tetanic force was measured from both SM muscles in each rat. The removed SM muscles were immunostained to detect regenerated axons and reinnervated and non-reinnervated motor endplates. The axon density was assessed using ImageJ software. Muscle force measurement demonstrated that NMEG-NMZ technique resulted in more optimal force recovery (82% of the control) as compared with the original NMEG procedure (67% of the control), in which the NMEG pedicle was implanted into the caudal endplate-free area of the target muscle. The mean muscle weight of the NME-NMZ reinnervated muscles was 89% of the contralateral control muscles. The mean count and area of the regenerated axons in the treated muscles were 76.8% and 75.6% of the contralateral controls, respectively. Double fluorescence staining showed that the regenerated axons from the implanted NMEG grew across the NMZ to innervate the denervated motor endplates in the target muscle. The majority (80%) of the denervated motor endplates in the target muscle regained motor innervation. Axonal sprouts and newly formed small endplates were also identified in the reinnervated muscles. Our results suggest that the NMZ of the target muscle is the best site for

NMEG implantation to obtain optimal axon-endplate connections and functional recovery and that NMEG-NMZ technique may become a viable means of muscle reinnervation in certain clinical situations.

Disclosures: **L. Mu:** None. **S. Sobotka:** None. **J. Chen:** None. **T. Nyirenda:** None.

Poster

675. Peripheral Nervous System Regeneration

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 675.25/C1

Topic: A.04. Transplantation and Regeneration

Support: NIH grant NS069844

Title: An evolutionarily conserved mechanism for cAMP elicited axonal regeneration involves direct activation of the dual leucine zipper kinase DLK

Authors: ***Y. HAO**[1], E. FREY[3], H. WONG[4], C. YOON[2], R. GIGER[2], L. HOLZMAN[4], A. DIANTONIO[3], C. COLLINS[1];
[1]MCDB, [2]CDB, Univ. of Michigan, Ann Arbor, MI; [3]Developmental biology, Washington Univ. in St. Louis, St. Louis, MO; [4]Univ. of Pennsylvania, Philadelphia, PA

Abstract: A broadly known method to stimulate the growth potential of axons is to elevate intracellular levels of cAMP, however the cellular pathway(s) that mediate this are not known. Here we identify the Dual Leucine-zipper Kinase (DLK, Wnd in *Drosophila*) as a critical target and effector of cAMP in injured axons. DLK/Wnd is thought to function as an injury 'sensor', as it becomes activated after axonal damage. Our findings in both *Drosophila* and mammalian neurons indicate that the cAMP effector kinase PKA is a conserved and direct upstream activator of Wnd/DLK. PKA is required for the induction of Wnd signaling in injured axons, and DLK is essential for the regenerative effects of cAMP in mammalian DRG neurons. PKA stimulates DLK by directly phosphorylating its activation loop and this regulation is independent of downstream JNK signaling. These findings link two important mediators of responses to axonal injury, DLK/Wnd with cAMP/PKA, into a unified and evolutionarily conserved molecular pathway for stimulating the regenerative potential of injured axons. Previous studies have implicated a role for DLK/Wnd is regulating the structure and arborization of presynaptic terminals. The addition of cAMP and PKA into this regulatory pathway suggests a mechanism that may be widely utilized to orchestrate structural changes at synapses.

Disclosures: **Y. Hao:** None. **E. Frey:** None. **H. Wong:** None. **C. Yoon:** None. **R. Giger:** None. **L. Holzman:** None. **A. Diantonio:** None. **C. Collins:** None.

Poster

675. Peripheral Nervous System Regeneration

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 675.26/C2

Topic: A.04. Transplantation and Regeneration

Support: DOD grant W81XWH-13-0-0078

Gift of Mr Mark Rubenstein

Title: The interaction of regenerating sensory and motor axons

Authors: ***T. M. BRUSHART**[1], F. KEBAISCH[2], R. WOLINSKY[2], R. SKOLASKY[2];
[1]Dept Orthopaedic Surgery, [2]Orthopaedics, Johns Hopkins, Baltimore, MD

Abstract: Preferential motor reinnervation (PMR) is the tendency for motor axons regenerating in mixed nerve to reinnervate muscle nerve and/or muscle. We have shown previously that PMR is enhanced when afferent axons are eliminated from the regenerate (Redett et al., 2005). We now use our organotypic model of nerve repair in vitro (Vyas et al., 2010) to explore interactions of regenerating sensory and motor axons that may underlie these findings.
Spinal cord slices in which motoneurons express YFP (thy1-YFP-H) and DRG explants from mice expressing RFP (ROSA mT/mG) were used to populate cultured segments of mouse femoral nerve with fluorescent axons. Nerves contained either green motor axons alone (motor group [M]; n=7) or both green motor and red sensory axons (sensory-motor group [SM]; n=6). These nerves were then transected and their axons grown out on unstructured collagen/laminin mats. After 5 days, axons were counted at each 0.25 mm increment from the nerve end. In the SM group we also quantified the length of each motor axon that was in direct contact with a sensory axon.
Significantly fewer motor axons regenerated to each distance interval from 0.25mm to 1.0 mm when these axons were accompanied by sensory axons (SM) than when they regenerated alone (M). Mean axon counts were: 0.25mm, SM=27, M=77, p=0.0; at 0.5mm, SM=10, M=70, p=0.0; at 0.75mm, SM=4, M=51, p=0.0002; at 1mm, SM=1, M=29, p=0.0016. To compare sensory and motor axon outgrowth within the SM group, counts were converted to percentages using the counts at 0.25 mm as 100%. The percentage of axons counted at 0.25mm that reached the 0.50, 0.75, and 1.0 mm levels was significantly higher for sensory axons (s) than for motor axons (m). At 0.5mm, s=84%, m=34%, p=0.0042; at 0.75mm: s=71%, m=12%, p=0.0012; at 1mm: s=43%,

m=2%, p=0.0005. Quantification of sensory and motor axon contact revealed that a mean of 81% of total motor axon length was in direct contact with sensory axons. These experiments quantify, for the first time, the interaction of two distinct populations of regenerating axon. They reveal that sensory axons extend more rapidly than motor axons, and thus precede them during the early stages of regeneration. Furthermore, motor axons adhere to sensory axons throughout most of their length. As a result of this previously unappreciated interaction, sensory axons inhibit motor axon growth. These findings suggest that defining and blocking the negative effects of sensory axons on their motor partners could enhance PMR.

Disclosures: T.M. Brushart: None. **F. Kebaisch:** None. **R. Wolinsky:** None. **R. Skolasky:** None.

Poster

675. Peripheral Nervous System Regeneration

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 675.27/C3

Topic: A.04. Transplantation and Regeneration

Support: NIH Grant P20GM0103423

NSF Grant DBI-1428210

Title: Neural dependence of fin regeneration in zebrafish

Authors: *N. W. KLECKNER[1], R. DOBBIN[2], H. LOEB[2], A. ESTRELLA[2], S. HOLMES[2]; [1]Biology/Neuroscience, [2]Neurosci., Bates Col., Lewiston, ME

Abstract: Most organisms have the ability to regenerate certain body parts. However, some vertebrates have a particularly extensive regenerative capacity. For example, some amphibian limbs and teleost fins can be regrown when lost, and both of these processes are well characterized. In both cases regeneration has been found to be dependent on the presence of nerves; once denervated, a limb loses its ability to regenerate following an amputation. In salamanders this dependence may be due to the release of anterior gradient proteins (nAg, in salamanders) by Schwann cells. While zebrafish (*Danio rerio)* tail regeneration is phenotypically well characterized, the molecular basis of the neural dependence of regeneration has not been adequately explored. The purpose of this study was to compare methods of denervation of the zebrafish caudal fin to assess the role of molecular factors that might play a role in fin regeneration. We explored *agr2* (a nAg orthologue), myelin basic protein and vascular endothelial growth factor expression in caudal fins that were denervated either manually or by

exposure to metronidazole or the ErbB (and Schwann cell) inhibitor AG-1478, and then amputated in 3-6 month old Nacre zebrafish. Denervation was verified by comparing anti-acetylated tubulin antibody labeling of caudal fin axons from treated and control fish. We found that the expression of *agr2* was upregulated in the caudal fin during regeneration in metronidazole treated fish, although not specifically in the blastema. Additionally, while mechanical denervations did not consistently remove neural input, exposure of fish to 5 mM metronidazole reduced axon density. Unexpectedly, metronidazole enhanced regeneration, suggesting that it changes expression of regenerative factors that are independent of neural input. These findings provide additional information on the identity of molecular factors involved in neural (or Schwann cell) dependence of fin regeneration. Conserved or contrasting pathways between regeneration of amphibian limbs and teleost fins may highlight why most vertebrates have such a limited ability to regenerate.

Disclosures: N.W. Kleckner: None. **R. Dobbin:** None. **H. Loeb:** None. **A. Estrella:** None. **S. Holmes:** None.

Poster

675. Peripheral Nervous System Regeneration

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 675.28/DP01 (Dynamic Poster)

Topic: A.04. Transplantation and Regeneration

Support: International Society for Invertebrate Neurobiology

Title: Nerve regeneration in Octopus vulgaris: possible involvement of stem progenitor cells and epigenetic regulation

Authors: *P. IMPERADORE[1], G. FIORITO[2];
[1]Assn. For Cephalopod Res. - Cephres, Napoli, Italy; [2]Biol. and Evolution of Marine Organisms, Stazione Zoologica Anton Dohrn, Napoli, Italy

Abstract: Aims
Octopus vulgaris, an invertebrate, is known since antiquities for remarkable regenerative abilities of body parts and tissues including arms and nervous tissue. A striking example of regeneration in this species is given by the pallial nerve, which is involved in the control of mantle muscle contraction facilitating breathing and of body patterning. After complete transection of this nerve, full recover of functions is observed and nerve regeneration achieved in relatively short time. Here we provide the first description of the biological machinery involved in the pallial nerve regeneration in the octopus, including the involvement of cellular types helping the process

and epigenetics regulation.

Methods and Results

Octopus pallial nerve (right side) is exposed and transected while the contralateral nerve served as control. After surgery, animals are recovered and behavior observed for 3, 7 and 14 days before humane-killing. Nerve samples are analyzed for gene and protein expression by qRT-PCR and immunohistochemistry.

After injury scar tissue forms between the two stumps of the injured nerve. Hemocytes contribute to the formation of the scar and remain highly proliferative during the whole process. The scar does not represent an inhibitory environment to regrowth, and fibers of the proximal stump start regenerating across it and toward the distal stump maintaining a disorganized orientation. Two weeks after injury a spike-like protrusion appears in this stump with connective tissue enwrapping the new forming axons. Degeneration is observed at the level of the distal stump. However, fourteen days post-surgery fibers organized in bundles are found in advanced regeneration.

Dedifferentiation of the connective tissue cells of the nerve is also observed in both stumps, particularly 7 days post lesion. Marked up-regulation in the expression of 'factors' controlling epigenetic modifications, such as Polycomb group proteins and DNA methyltransferases, is also observed.

Conclusions

Active role of octopus hemocytes in the pallial nerve regeneration is found such as in scar tissue formation, debris removal and axonal regrowth. In addition, cells of the connective tissue drive nerve fibers regeneration facilitating their correct orientation; these also appear to dedifferentiate to a neural progenitor/stem cell state during the process.

Epigenetic factors have been identified for the first time in the *O. vulgaris* and up-regulation of their expression has been highlighted during the regenerative process compared to control nerves.

Disclosures: P. Imperadore: None. **G. Fiorito:** None.

Poster

675. Peripheral Nervous System Regeneration

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 675.29/C4

Topic: A.04. Transplantation and Regeneration

Support: KAKEHIN 25293137

KAKEHIN 26670291

Title: Involvement of Na^+/K^+-ATPase in peripheral nerve regeneration via lactate signaling in sciatic nerve transection-regeneration model

Authors: N. H. TU[1], *S. ITO[2], M. SHINJI[1], K. TAYO[1], P. MINH VUONG[1];
[1]Dept. of Med. Chem., Kansai Med. Univ., Hirakatashi - Osaka, Japan; [2]Med. Chem., Kansai Med. Univ., Hirakata-shi, Osaka, Japan

Abstract: Peripheral nerve is not always regenerated fully after injury. Incomplete regeneration causes functional loss and persistent pain. To prevent these consequences, elucidation of mechanisms for nerve regeneration is crucial. We previously established a sciatic nerve transection-regeneration model and demonstrated that functional recovery was delayed in mice lacking Nax, a Na^+ concentration-sensitive Na^+ channel. Endothelin receptor type B functionally coupled with Na_x and increased sodium influx to Schwann cells, which in return enhanced lactate release. The released lactate was then uptaken to axons to accelerate nerve regeneration (Unezaki et al., Eur. J. Neurosci. 39:720-729, 2014). Nax is known to interact with Na^+/K^+-ATPase leading to lactate production in the brain. In this work, we investigated the role of Na^+/K^+-ATPase in peripheral nerve regeneration in our model and applied ouabain, a Na^+/K^+-ATPase inhibitor, to the cutting site of the sciatic nerve for 4 weeks with an osmotic mini-pump. While functional recovery started at the 5th week after nerve transection and completed by the 7th week, ouabain delayed the functional recovery by 1 week as compared to the vehicle control. Lactate reversed the inhibitory effect of ouabain and a monocarboxylate transporter (MCT) inhibitor, significantly delayed functional recovery. Using primary DRG cell culture, we tested the effect of ouabain, lactate, and MCT inhibitor on neurite outgrowth enhanced by nerve growth factor (NGF). NGF accelerated neurite outgrowth by 1.9 times as compared with the vehicle, but ouabain reversed the stimulatory effect of NGF in a concentration-dependent manner. Lactate itself also increased neurite outgrowth 2 times as compared to the control, but inhibition of lactate transport by MCT 4 inhibitor suppressed neurite outgrowth. Collectively, Na^+/K^+-ATPase has a role in peripheral nerve regeneration via lactate signaling.

Disclosures: **N.H. Tu:** None. **S. Ito:** None. **M. Shinji:** None. **K. Tayo:** None. **P. Minh Vuong:** None.

Poster

676. Cytoskeletal Mechanisms Underlying Axon Outgrowth and Guidance

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 676.01/C5

Topic: A.05. Axon and Dendrite Development

Support: NIH Grant NS078030

Title: Nerve growth factor induces mitochondrial fission which is required for axon branching

Authors: *L. ARMIJO WEINGART, A. KETSCHEK, R. SAINATH, A. PACHECO, G. GALLO;
Temple Univ., Philadelphia, PA

Abstract: Nerve Growth Factor (NGF) induces branching through activation of phosphoinositide 3-kinase (PI3K). Recently, mitochondria have emerged as major determinants of the sites of axon branching. NGF treatment decreased the length and increased the number of axonal mitochondria labeled with mitotracker green after 15 min of acute treatment, indicative of fission. Consistently, live imaging of mitochondria following 5 minutes of NGF treatment revealed mitochondria fission. Direct activation of PI3K using a cell permeable peptide in the absence of NGF copied the effects of NGF. Conversely, inhibition of PI3K using LY294002 blocked the effects of NGF on mitochondria. Pharmacological and peptide-mediated inhibition of dynamin related protein 1 (Drp1), a required component of mitochondria fission, blocked NGF induced axon branching. Live imaging of axons in the presence of NGF revealed that EYFP-Drp1 accumulated at sites of mitochondria fission and endogenous Drp1 was detected in axons through immunocytochemistry. NGF promoted phosphorylation of Drp1 at the activating site S616 trough ERK activation independently of the PI3K pathway. Additionally, inhibition of ERK signaling blocked the effect of NGF in mitochondria fission and Drp1 accumulation along the mitochondria. Inhibition of actin polymerization using Latrunculin-A also blocked the effect of NGF in mitochondria fission and Drp1 accumulation. Furthermore, live imaging of axons indicates that actin patches strongly co-localize with sites of mitochondria fission. Finally, we found that Brain-derived neurotrophic factor and Neurotrophin-3 also decrease the length and increase the number of mitochondria. Collectively, these observations indicate that NGF mediates phosphorylation of Drp1 through ERK signaling, and drives mitochondria fission through the activation of both the ERK and PI3K pathways. As PI3K signaling is not involved in Drp1 phosphorylation, PI3K might contribute to fission through regulation of the actin cytoskeleton independently of changes in Drp1 activity.

Disclosures: L. Armijo Weingart: None. **A. Ketschek:** None. **R. Sainath:** None. **A. Pacheco:** None. **G. Gallo:** None.

Poster

676. Cytoskeletal Mechanisms Underlying Axon Outgrowth and Guidance

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 676.02/C6

Topic: A.05. Axon and Dendrite Development

Support: NIH Grant NS078030 to G.G.

Morton Cure for Paralysis Foundation to G.G.

Shriners Hospitals Fellowship to R.S.

Title: Chondroitin sulfate proteoglycans inhibit axon branching by impairing the mitochondria-dependent local regulation of actin cytoskeletal dynamics and axonal protein synthesis

Authors: *R. SAINATH, A. KETSCHEK, L. GRANDI, G. GALLO;
Shriners Hosp. Pediatric Res. Ctr., Temple Univ., Philadelphia, PA

Abstract: Chondroitin sulfate proteoglycans (CSPGs) are extracellular matrix components that regulate aspects of axon development and regeneration. CSPGs inhibit the formation of axon collateral branches. Branching requires the regulation of the axonal cytoskeleton and mitochondria are important components of this mechanism of branching. Here we report that CSPGs depolarize the membrane potential of axonal mitochondria, which impairs the dynamics of the axonal actin cytoskeleton (e.g., axonal actin patches) and decreases the formation and duration of axonal filopodia, the first step in the mechanism of branching. The effects of CSPGs on actin cytoskeletal dynamics are specific to axon segments populated by mitochondria. In contrast, CSPGs do not affect the microtubule content of axons, or the localization of microtubules into axonal filopodia, a required step in the mechanism of branch formation. We also report that CSPGs decrease the mitochondria-dependent axonal translation of cortactin, an actin associated protein involved in branching. Finally, the inhibitory effects of CSPGs on axon branching, actin cytoskeletal dynamics and the axonal translation of cortactin were reversed by culturing neurons with acetyl-L-carnitine, which promotes mitochondrial respiration. Collectively these data indicate that CSPGs impair mitochondrial function in embryonic sensory axons, an effect which contributes to the inhibition of axon branching.

Disclosures: **R. Sainath:** None. **A. Ketschek:** None. **L. Grandi:** None. **G. Gallo:** None.

Poster

676. Cytoskeletal Mechanisms Underlying Axon Outgrowth and Guidance

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 676.03/C7

Topic: A.05. Axon and Dendrite Development

Support: NIH grant NS044916

NIH grant NS069688

Adelson medical research foundation

Title: αII-spectrin-dependent cytoskeletons are essential for axon function, domain assembly and integrity

Authors: *Y. HUANG[1], C. ZHANG[1], D. ZOLLINGER[2], J. LALONDE[1], C. LETERRIER[3], J. NOEBELS[1], M. RASBAND[1];
[1]Baylor Col. of Med., Houston, TX; [2]UCSF, San Francisco, CA; [3]Aix Marseille Univ., Marseille, France

Abstract: Spectrins are a family of cytoskeletal proteins that provide structural support of the cell membrane, link membrane-associated proteins to actin and serve as platforms for cell signaling. Spectrins consist of α and β subunits, forming heterotetramers to function as a complex. Among the spectrins, αII-spectrin is the only α-spectrin expressed in the nervous system. αII-spectrin is also implicated in a variety of neurological disorders. Recently, we found that αII-spectrin forms a periodic cytoskeleton and interacts with βIV-spectrin at axon initial segments (AIS) and nodes of Ranvier. To investigate the functions of αII-spectrin-dependent cytoskeletons, we generated conditional knockout (cko) mice. Loss of αII-spectrin in the central nervous system (CNS) causes profound neurological phenotypes including seizures, aberrant cortical lamination, AIS fragmentation, massive neurodegeneration and perinatal lethality. To more specifically interrogate spectrin functions in axons, we generated peripheral sensory neuron specific αII-spectrin cko mice using *advillin-cre.* We found that large diameter axons preferentially degenerate. By immunostaining, the injury marker ATF3 is observed in dorsal root ganglia (DRG) neurons in cko mice beginning at P10 and increasing with age. Consistent with EM results, $ATF3^+$ neurons are mostly large diameter neurons. The preferential degeneration of large diameter neurons caused ataxia due to deficits in proprioception, while nociception remains unaffected. Mutant mice have fewer nodes of Ranvier and sodium channel intensity at nodes is significantly decreased. Paranodal junctions are extensively disrupted. Axon degeneration and disrupted nodes of Ranvier caused decreased nerve conduction velocity in cko mice. Thus, neuronal αII-spectrin is crucial for proper axon function, node of Ranvier assembly and axon integrity. **Support:** This reseach is funded by NIH grant NS044916, NS069688 and the Adelson medical research foundation.

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Poster

676. Cytoskeletal Mechanisms Underlying Axon Outgrowth and Guidance

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 676.04/C8

Topic: A.05. Axon and Dendrite Development

Support: TWU Research Enhancement Program

Title: Identifying signaling cascades involved in growth and cell clustering of functional, non-prenylatable RhoA and Rac1

Authors: *N. G. RAUT, J. M. REDDY, D. L. HYNDS;
Biol., Texas Women's Univ., Denton, TX

Abstract: The Rho guanine triphosphatase (GTPase) are highly characterized and GTPase proteins that act as molecular switches operating between an active GTP-bound state and an inactive GDP-bound state. These proteins play a pivotal role in neuronal differentiation and affect neurite outgrowth, axonal guidance, cell migration, cytokinesis and endocytosis. RhoA promotes assembly of focal adhesion complexes and formation of stress fibers. RhoA regulates the organization of actin cytoskeleton and several other cellular functions in response to the extracellular signals. On the other hand, Rac1 stimulates assembly of multimolecular focal complexes at plasma membrane, induces the peripheral actin accumulations, regulates membrane protrusions, membrane ruffling and formation of lamellipodia and filopodia. The interaction between RhoA and Rac1 is not well explored, though they are thought to be antagonist to each other. Both require prenylation for membrane localization, though active forms of both have been found in other cellular compartments (GTP-bound Rac1 in the cytosol and GTP-RhoA primarily in the cytosol and nucleus). We designed non-prenylatable Rac1 and RhoA constructs to test how inhibiting prenylation affects signaling cascades involved in neurite outgrowth. Western blot analysis after transfection with the wild-type RhoA decreased cofilin in the cytosol compared to that associated with membranes. ERK and JNK phosphorylation was increased in cytosol when cells were transfected with non-prenylatable RhoA or Rac1. We have found transfection of these constructs in rat cortical neurons increase neurite outgrowth (for non-prenylatable RhoA) and neurite formation (for non-prenylatable Rac1). Both retained the ability to be made active independent of membrane targeting by prenylation. With emerging evidence of differential activation of these Rho GTPases based on their subcellular localization, elucidating the signaling cascades of the active GTPases may identify the distinct functions of these GTPases in the cytosol and can be used as novel targets to facilitate axon regeneration in traumatic or degenerative neurological conditions. This research was supported by the TWU Department of Biology and grants from the TWU Research Enhancement Program.

Disclosures: **N.G. Raut:** None. **J.M. Reddy:** None. **D.L. Hynds:** None.

Poster

676. Cytoskeletal Mechanisms Underlying Axon Outgrowth and Guidance

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Topic: A.05. Axon and Dendrite Development

Support: KAKENHI 15K14889

KAKENHI 22115009

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KAKENHI 15H04263

KAKENHI 16K14559

KAKENHI 15K14889

Uehara Memorial Foundation

Title: RacGAP α-chimaerin is required to establish spinal midline barrier for proper corticospinal projection

Authors: *S. KATORI[1], S. ITOHARA[2], T. IWASATO[1,3];
[1]Natl. Inst. of Genet., Mishima, Shizuoka, Japan; [2]RIKEN Brain Sci. Inst., Wako, Saitama, Japan; [3]Dept. of Genet., SOKENDAI (The Grad. Univ. for Advanced Studies), Mishima, Shizuoka, Japan

Abstract: In the developing central nervous system, "midline barrier (MB)", which consists of radial glia and their processes expressing repulsive guidance molecules, plays a pivotal role for midline axon guidance. However, mechanisms for MB formation have remained obscure. We here describe that Rac-specific GTPase activating protein (RacGAP) α-chimaerin, which we and others previously identified as a key downstream effector of EphA4 forward signaling, is required for MB establishment. We generated spinal cord-specific α-chimaerin knockout mice and found that these mice had numerous “cracks” in dorsal spinal cord MB, which expresses ephrinB3 to prevent EphA4(+) axons from midline crossing. Through these cracks, corticospinal axons expressing EphA4 aberrantly crossed the midline. During embryonic development of normal mice, EphA4(+) cells are localized in the vicinity of, but not within, the spinal cord midline. In contrast, in α-chimaerin knockout mice, extensive midline accumulation of spinal EphA4(+) cells was observed, and MB cracks emerged around these cells. We found similar phenotypes in EphA4-deficient mice. Our results suggest that spinal cord α-chimaerin repeals juxta-midline EphA4(+) cells from ephrinB3(+) MB in the spinal cord and plays a critical role

for establishing intact spinal MB. Here we shed light on an MB establishment mechanism, in which ephrinB3-EphA4-α-chimaerin signaling-mediated cell repulsion could be involved.
(Refs) 1. Iwasato, T.et al. Cell 130, 742-753. (2007).
2. Borgius, L., Nishimaru, H. et al., J. Neurosci. 34, 3841-3853. (2014).
3. Iwata, R.et al., Cell Rep. 8, 1257-1264. (2014).
4. Iwata, R. et al., J. Nerurosci. 35, 13728-44. (2015).

Disclosures: S. Katori: None. **S. Itohara:** None. **T. Iwasato:** None.

Poster

676. Cytoskeletal Mechanisms Underlying Axon Outgrowth and Guidance

Location: Halls B-H

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Program#/Poster#: 676.06/C10

Topic: A.05. Axon and Dendrite Development

Title: RACK1 regulates point contacts and local translation in growth cones

Authors: *L. J. KERSHNER[1], K. WELSHHANS[2];
[1]Dept. of Biol. Sci., [2]Dept. of Biol. Sciences, Sch. of Biomed. Sci., Kent State Univ., Kent, OH

Abstract: In the developing nervous system, select mRNAs are transported to and locally translated within growth cones. Formation of appropriate connectivity in the developing nervous system is dependent on local translation within axonal growth cones, but the specific locations and molecular mechanisms underlying this process are not well understood. We have previously shown that local translation of β-actin mRNA within growth cones is necessary for appropriate axon guidance and is dependent on receptor for activated C kinase (RACK1). RACK1 is a multi-functional ribosomal scaffolding protein that can interact with a number of signaling molecules concurrently through its 7WD repeats. In response to stimulation with brain-derived neurotrophic factor (BDNF), phosphorylation of RACK1 facilitates the local translation of β-actin mRNA. We recently found that RACK1 is localized to point contacts, suggesting that local translation may be regulated at point contacts, adhesion sites important for axonal pathfinding. Thus, here we investigate whether local translation occurs at point contacts and examine the role of RACK1 in the regulation of point contact dynamics. First, we examined the location of components of the local translation complex relative to point contacts under both basal and growth factor stimulated conditions in cortical neurons of embryonic day 17 C57BL/6J mice. Indeed, both β-actin mRNA and RACK1 colocalize with point contacts, and this colocalization increases following BDNF stimulation. This suggests that local translation is regulated at point contacts. Additionally, RACK1 is necessary for point contact formation, and the density of point contacts within growth cones increases following BDNF stimulation in a RACK1 dependent manner. Phosphorylation of

RACK1 is also required for the BDNF-induced increase in point contact density. Furthermore, live cell experiments using total internal reflection fluorescence (TIRF) microscopy demonstrate a role for RACK1 in the regulation of point contact dynamics. Finally, we demonstrate that RACK1 is vital for functional aspects of neuronal development. Axonal growth and growth cone spreading require both RACK1 expression and phosphorylation. Taken together, these data suggest that point contacts are a targeted site of local translation within growth cones, and that RACK1 is critical to the formation of point contacts, the local translation process, and appropriate neuronal development. These data provide further insight into how and where local translation is regulated within growth cones, and thereby leads to appropriate connectivity formation in the developing nervous system.

Disclosures: L.J. Kershner: None. **K. Welshhans:** None.

Poster

676. Cytoskeletal Mechanisms Underlying Axon Outgrowth and Guidance

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 676.07/C11

Topic: A.05. Axon and Dendrite Development

Title: Kinesin-1 is required for cannabinoid-induced axonal development through the axonal transport of CB1 receptor.

Authors: *T. M. SAEZ[1,2], M. OTERO[1], G. OUBIÑA[2], L. E. CROMBERG[1], M. ALLOATTI[1], V. M. POZO DEVOTO[1], D. GELMAN[2], T. L. FALZONE[1,2];
[1]Inst. De Biología Celular Y Neurociencia, Ciudad Autónoma De Buenos Aires, Argentina; [2]Inst. de Biología y Medicina Exptl., Ciudad Autónoma de Buenos Aires, Argentina

Abstract: During development, axonal navigation through the intricate architecture of the brain depends on the proper presentation and positioning of guidance receptors, which allow for the correct reading of external clues. Receptors for axonal growth and guidance are shifted to axons and localized in growth cones, where they are activated by attractive or repulsive guidance cues resulting in axonal pathfinding decisions. However, little is known about the crucial mechanisms controlling the proper trafficking of these receptors involved in neural circuits wiring. The endocannabinoid (eCB) system has been identified as an important regulator of axonal outgrowth and pathfinding. eCBs mediate the motility and directional turning of axons by activating type 1 cannabinoid receptor (CB1R) in the axonal growth cone. Although cargo delivery mediated by molecular motors is essential in developing neurons, the mechanism underlying the directional axonal transport of CB1R remains basically unknown. To test the hypothesis that CB1R delivery to the growth cone depends on kinesin-1 mediated axonal transport, we used mice lacking the

kinesin light chain 1 (*klc1*) subunit of the anterograde motor kinesin-1. We performed L1-NCAM staining and axon-tracing experiments in developing *klc1*-/- brains and found pathfinding defects in corticothalamic and thalamocortical axonal tracts, which resemble those in CB1R knockout mice. Using live imaging of fluorescent CB1R tagged vesicles in wildtype and *klc1*-/- neurons, we revealed the dependency of Kinesin-1 in CB1R transport towards the axonal growth cone. Next, to assess the role of kinesin-1 on CB1R-mediated axonal development, primary cortical cultures of wildtype and *klc1*-/- neurons were treated with pharmacologic modulators of the CB1R (WIN55-212,22, ACEA and AM251). We demonstrated that kinesin-1 is required for the correct axonal growth cone remodeling and outgrowth-induced by CB1R agonist and antagonist. Altogether, our results suggest that kinesin-1-mediated axonal transport of CB1R is required for a normal eCB signaling and is also required for proper axonal pathfinding.

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Poster

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Title: DCLK1 phosphorylates the microtubule-associated protein MAP7D1 to promote axon elongation in cortical neurons

Authors: *H. KOIZUMI[1], K. TOGASHI[1], J. GLEESON[2,3], K. EMOTO[1];
[1]Dept. of Biol. Sci., The Univ. of Tokyo, Tokyo, Japan; [2]Lab. of Pediatric Brain Dis., The Rockefeller Univ., New York, NY; [3]Howard Hughes Med. Inst., New York, NY

Abstract: Doublecortin-like kinase 1 (DCLK1) is a neuronal serine-threonine protein kinase that is a closely related family protein of Doublecortin (DCX) originally identified as a causative gene product of human cortical malformation. DCLK1 contains the microtubule-binding domain at N-terminus that has 75% amino acid identity to DCX and the protein kinase domain at C-terminus. In mice, targeted disruption of both *Dclk1* and *Dcx* results in severe defects in cortical

lamination and the formation of axonal projections. Thus DCLK1 functions together with DCX in multiple stages of neural circuit formation including neuronal migration and axon growth. DCLK1 is suggested to play these roles in part through its kinase activity, yet the kinase substrates of DCLK1 remain largely unknown. Here we have identified mouse MAP7D1 (microtubule-associated protein 7 domain containing 1) as a novel substrate of DCLK1. miRNA-mediated knockdown of MAP7D1 in cortical layer 2/3 pyramidal cells resulted in impaired callosal axon elongation. We have further identified MAP7D1 serine 315 (Ser 315) as a DCLK1-induced phosphorylation site, and the expression of a phosphomimetic mutant of MAP7D1 fully rescues the impaired callosal axon elongation caused by *Dclk1* knockdown. These data suggest that DCLK1 phosphorylates MAP7D1 on Ser 315 to facilitate axon elongation of cortical neurons.

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Poster

676. Cytoskeletal Mechanisms Underlying Axon Outgrowth and Guidance

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Topic: A.05. Axon and Dendrite Development

Support: 1F31NS093748-01A1

Title: A dynein-based mechanism, repurposed from neuronal migration, establishes and preserves microtubule organization in the axon

Authors: *A. RAO[1], M. BLACK[2], E. CRAIG[3], K. MYERS[4], P. BAAS[1];
[1]Drexel Univ., Philadelphia, PA; [2]Anat. and Cell Biol., Temple Univ. Sch. of Med., Philadelphia, PA; [3]Physics, Central Washington Univ., Ellensburg, WA; [4]Dept. of Biol. Sci., Univ. of the Sci., Philadelphia, PA

Abstract: We propose that dynein-driven forces manifest in different ways at different stages of neuronal life, depending on the capacity of the MTs to slide. MT sliding is limited by the attachment of MTs to the centrosome, as well as their length, with longer MTs being less able to slide. In migratory neurons, it has long been believed that all functionally relevant MTs are attached to the centrosome, which would render them unable to slide relative to one another. However, we were able to document a limited amount of MT sliding in the leading process of cultured cerebellar migratory neurons, with treatment with a drug that inhibits MT sliding resulting in an impairment of the trajectory of migration. By knocking down in cultured rat cerebellar granule neurons the expression of ninein, a centrosomal protein responsible for

anchoring MTs to the centrosome, we increased the number of centrosome-unattached MTs and observed an increase in MT sliding. Concomitantly, migration was severely compromised and the leading process became longer, reminiscent of the phenotype of an axon-bearing post-migratory neuron. Next, we pursued the idea that in a bona fide axon where MTs appear in a variety of lengths that dynein-based forces are repurposed to establish and preserve the nearly uniform plus-end-out polarity pattern of the MT array. Exposure of cultured rat sympathetic neurons to the Ciliobrevin D, a small molecule dynein inhibitor, for various windows of time resulted in a dose-dependent appearance of greater numbers of minus-end-distal MTs in the axon. In addition, MT transport events were reduced in frequency in both the anterograde and retrograde directions. The MT movements that did occur in the presence of the drug showed an increase in abrupt pausing of movements and directional changes in the movements, as well as abnormal transport rates. These effects are consistent with a mechanism whereby dynein-based forces propel MTs with plus-end-out anterogradely down the axon, while clearing MTs with minus-end-out back to the cell body. These two sets of data, on neurons of different stages of development, demonstrate how dynein-driven forces on MTs can be repurposed to orchestrate different neuronal phenotypes.
2234/2300

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Poster

676. Cytoskeletal Mechanisms Underlying Axon Outgrowth and Guidance

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

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Topic: A.05. Axon and Dendrite Development

Support: TWU Department of Biology

Title: Regulation of mutant Arp3 in branch actin polymerization

Authors: P. DHANJANI, 76204[1], S. HALDAR[1], A. MAHADIK[1], B. BECK, 78758-4497[2,3], *D. L. HYNDS[1];
[1]Texas Woman's Univ., Denton, TX; [2]Texas Woman's Univ., Austin, TX; [3]Texas Academic Computing Ctr., Univ. of Texas at Austin, Austin, TX

Abstract: The actin related protein (Arp) 2/3 complex is a seven subunit complex that is required for the nucleation of branched actin filaments for the development of neurons. Within a cell, several proteins tightly regulate the activity of this complex but how these proteins

modulate the activity of the Arp 2/3 complex is still unclear on a structural basis. Structural modeling identified several mutations that are important in maintaining the active structure of the Arp 2/3 complex. We constructed these mutant and are using them as tools to decipher the structure-based functional relationships governing Arp 2/3 mediated nucleation of actin. The current work involves understanding the effect of different regulators (rac1 inhibitor, arp 2/3 complex inhibitor, and profilin) on actin polymerization activity of constitutively active Arp3 mutant in neuroblastoma cells. The results from this study will help in understanding the structural regulation of Arp3 protein in the development of neurons and, hence, in the regeneration of the neuronal network at the site of spinal cord injury.

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Poster

676. Cytoskeletal Mechanisms Underlying Axon Outgrowth and Guidance

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Topic: A.05. Axon and Dendrite Development

Support: NRF Grant/2015R1C1A1A02036674

Title: Axonal mRNA dynamics in live hippocampal neurons

Authors: *B. LEE[1], S. BANG[2], S. LEE[2], N. JEON[2], H. PARK[1];
[1]Physics and Astronomy, Seoul Natl. Univ., Seoul/Gwanak-gu, Korea, Republic of; [2]Inst. of Advanced Machinery and Design Seoul Natl. Univ., Div. of WCU (World Class University) Multiscale Mechanical Design Sch. of Mechanical and Aerospace Engin., Seoul, Korea, Republic of

Abstract: Localization of mRNA and protein synthesis is critical for axonal guidance and regeneration. Yet it is not clearly understood how mRNA localization is regulated in axons. Using a transgenic mouse that expresses fluorescently labeled beta-actin mRNA (Park, Lim et al. 2014) , we investigated the dynamics of endogenous beta-actin mRNA in axons. By culturing hippocampal neurons in a microfluidic device which allows separation of axons from cell bodies, it is possible to track β-actin mRNA motions in live axons. We observed high-frequency random oscillating movements of β-actin mRNA, which were clearly different from the movements in the dendrites. In addition, we found several mRNAs localized at axonal varicosities where synapses typically occur. We modeled mRNA dynamics in axons using a theoretical random walk model, Orstein-Uhlenbeck process that has a specific destination. This study suggests a

biophysical mechanism of mRNA transport and localization in axons, which will have important implications for axon regeneration.
Park, H. Y., H. Lim, Y. J. Yoon, A. Follenzi, C. Nwokafor, M. Lopez-Jones, X. H. Meng and R. H. Singer (2014). "Visualization of Dynamics of Single Endogenous mRNA Labeled in Live Mouse." Science **343**(6169): 422-424.

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Poster

676. Cytoskeletal Mechanisms Underlying Axon Outgrowth and Guidance

Location: Halls B-H

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Program#/Poster#: 676.12/C16

Topic: A.05. Axon and Dendrite Development

Title: Dynamics of cortical neuronal growth, transport and motility associated with cell trauma assessed by quantitative phase imaging (QPI)

Authors: Y. J. LEE[1], P. CINTORA[1], M. E. KANDEL[2], *C. A. POPESCU[3];
[1]Neurosci. program, [2]Electrical and Computer Engin., [3]Bioengineering, UIUC, Urbana, IL

Abstract: Characteristic neuronal changes occur following Traumatic Brain Injury (TBI) including axonal injury which is comprised of axonal swelling, degeneration and the loss of synapses, alterations in dendrites (reduction in dendritic spine density) and ultimately neuronal cell death. We speculate that changes in transport rate and deterministic axonal growth are altered following mechanical injury induced cell trauma. Advances in quantitative phase imaging (QPI) techniques allow unlabeled live biological specimens to be imaged with sub-nanometer sensitivity and diffraction limited resolution. The recently developed technique called Spatial Light Interference Microscopy (SLIM) has been useful in discovering the emergent behavior in human neuronal networks and the intercellular dynamics and SLIM 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 dynamic structural changes over long time scales. We use this imaging technique to measure changes in axon growth cone dynamics (sprouting and extension), neuronal extension transport rates, deterministic motility and we provide 3D cellular topography maps at high-resolution, in live cortical neurons over time, following mechanical cell trauma induced by a fluid percussion injury device.

Disclosures: **Y.J. Lee:** None. **P. Cintora:** None. **M.E. Kandel:** None. **C.A. Popescu:** None.

Poster

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Title: Mechanistic study of neurite outgrowth stimulated by the amyloid precursor protein interactor FE65

Authors: *W. LI, H. CHEUNG, K.-F. LAU;
Sch. of Life Sci., The Chinese Univ. of Hong Kong, Hong Kong, Hong Kong

Abstract: Alzheimer's disease is a devastating neurodegenerative disorder that affecting millions of people worldwide. Severe neurite degeneration is observed in Alzheimer's disease which is suggested to be associated with cognitive decline in the disease. FE65 is a brain-enriched multi-domain adaptor protein which is shown to interact with the Alzheimer's disease amyloid precursor protein (APP) and to alter APP metabolism. Moreover, recent evidence reveals that FE65 is also involved in other neurophysiological processes including neurite development. However, the mechanism(s) by which FE65 stimulates neurite outgrowth is still largely unknown. As an adaptor protein, FE65 functions in recruiting various interactors to form biological complexes. Hence, identification of the full complement of FE65 interacting proteins is an important step for improving our understanding of FE65 in various processes including neurite development. In this project, we have identified several small GTPases interact with FE65 including ARF6 and Rac1 and their corresponding regulators. ARF6-Rac1 signaling has been implicated in neurite outgrowth via regulation of cytoskeleton remodeling. In fact, FE65 stimulates neurite outgrowth in primary neurons via activation of ARF6 and its downstream GTPase Rac1. Additionally, we also found that the regulators of ARF6 and Rac1 are required for FE65-mediated neurite extension. Our work suggests that FE65 functions as an adaptor to recruit

various small GTPase and their regulators to form functional complexes to regulate neurite outgrowth. Since neurite degeneration is observed in Alzheimer's disease, strategies that attenuate neurite loss and/or stimulate neurite outgrowth may represent a promising direction for therapeutic development for the disease. Our work shed important light on the role of FE65 in neurite outgrowth.

Disclosures: W. Li: None. **H. Cheung:** None. **K. Lau:** None.

Poster

676. Cytoskeletal Mechanisms Underlying Axon Outgrowth and Guidance

Location: Halls B-H

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Topic: A.05. Axon and Dendrite Development

Support: DFG, CRC 1080

ERC-AG "LiPsyD"

Title: Precise somatotopic thalamocortical axon guidance depends on LPA-mediated PRG-2/Radixin signaling

Authors: J. CHENG[1], S. SAHANI[1], T. HAUSRAT[4], J.-W. YANG[2], H. ENDLE[1], X. LIU[1], Y. LI[1], R. BÖTTCHE[1], K. RADYUSCHKIN[3], A. HOERDER-SUABEDISSEN[5], Z. MOLNAR[5], P.-H. PROUVOT[1], T. TRIMBUCH[6], O. NINNEMANN[6], J. HUAI[1], W. FAN[1], B. VISENTIN[7], R. SABBADINI[7], A. STROH[1], H. J. LUHMANN[2], M. KNEUSSEL[4], R. NITSCH[1], *J. VOGT[8]; [1]Inst. for Microanatomy and Neurobio., [2]Inst. of Physiol., [3]Focus Program Translational Neurosci., Univ. Medicine, Johannes-Gutenberg-University, Mainz, Germany; [4]Ctr. for Mol. Neurobio. Hamburg, ZMNH, Inst. for Mol. Neurogenetics, Univ. Med. Ctr. Hamburg-Eppendorf, Hamburg, Germany; [5]Dept. of Physiology, Anat. and Genet., Univ. of Oxford, Oxford, United Kingdom; [6]Inst. for Cell Biol. and Neurobio., Charite, Berlin, Germany; [7]Lpath, San Diego, CA; [8]Univ. Medicine, Johannes-gutenberg-University, Mainz, Germany

Abstract: Precise connection of thalamic barreloids with their corresponding cortical barrels is critical for processing of vibrissal sensory information. Here, we show that the phospholipid interacting molecule PRG-2 plays an important role in thalamocortical axon guidance. Developing PRG-2$^{-/-}$ thalamocortical fibers prematurely entered the cortical plate eventually innervating non-corresponding barrels. This misrouting was due to lost axonal sensitivity towards lysophosphatidic acid (LPA), which failed to repel PRG-2-deficient thalamocortical fibers. PRG-2 electroporation in the PRG-2$^{-/-}$ thalamus was able to restore the aberrant cortical

innervation. We identified radixin (RDX) as a PRG-2 interaction partner in thalamocortical fibers, and showed that RDX accumulation in axon growth cones and its phosphorylation upon LPA stimulation was dependent on PRG-2. *In vivo* recordings and whisker-specific behavior tests demonstrated sensory discrimination deficits in PRG-2$^{-/-}$ animals. Our data show that bioactive phospholipids and PRG-2 are critical for axon guidance of thalamic fibers to their proper cortical termination field.

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Poster

676. Cytoskeletal Mechanisms Underlying Axon Outgrowth and Guidance

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Program#/Poster#: 676.15/C19

Topic: A.05. Axon and Dendrite Development

Support: DFG SFB815

Title: Cyclic gmp dependent protein kinase 1 guides axonal regeneration after sciatic nerve injury

Authors: ***L. VALEK**[1], A. HÄUSSLER[1], S. DRÖSE[2], K. SCHRÖDER[3], I. TEGEDER[1]; [1]Clin. Pharmacol., [2]Anaesthesiology, [3]Cardiovasc. Physiol., Univ. Hosp. Frankfurt, Frankfurt, Germany

Abstract: Cyclic GMP-dependent protein kinase 1 (PKG1) essentially contributes to nociceptive long-term potentiation in the spinal cord, and consequently, PKG inhibition or knockout in sensory neurons of the dorsal root ganglia (SNS-PKG1$^{-/-}$) reduces inflammatory nociception in mice, but unexpectedly, PKG1 deficiency in SNS-PKG1$^{-/-}$ mice provided no protection against nerve injury evoked neuropathic nociception. The result suggested specific repair functions of PKG1 that are necessary for regeneration and indeed, these functions were impaired after nerve injury because PKG1 transcription, redox-sensitive dimerization/auto-activation and axonal transport were reduced or blocked. Primary neurons of adult SNS-PKG1$^{-/-}$ mice showed enhanced branching and outgrowth due to a reduction of growth cone collapse evoked by oxidation events that occur physiologically at the growth cones and branching points in response to repulsion cues. Consequently, PKG1 deficient outgrowing axons *in vivo* failed to find the path

through a nerve lesion. At the molecular level, PKG1 deficiency impaired cofilin phosphorylation and hence actin dynamics essential for growth cone collapse evoked e.g. by semaphorin 3a. Enhanced outgrowth but defective path-finding may increase aberrant sprouting thereby outweighing spinal mechanisms of pain attenuation provided by PKG1-deficiency in inflammatory conditions and PKG1 inhibition after nerve injury may therefore impair endogenous repair mechanisms.

Disclosures: **L. Valek:** None. **A. Häussler:** None. **S. Dröse:** None. **K. Schröder:** None. **I. Tegeder:** None.

Poster

676. Cytoskeletal Mechanisms Underlying Axon Outgrowth and Guidance

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 676.16/C20

Topic: A.05. Axon and Dendrite Development

Title: BIG1 is required for the survival of deep layer neurons and the thalamocortical connectivity in developing brain.

Authors: ***J.-J. TEOH**[1], T. IWANO[2], M. KUNII[1], A. HARADA[1];
[1]Grad. Sch. of Med., Osaka Univ., Suita-Shi, Japan; [2]Grad. Sch. of Med. and Engin., Univ. of Yamanashi, Yamanashi, Japan

Abstract: As embryonic neocortex develop, radial glial cells in ventricular zone (VZ) start to produce postmitotic neurons around embryonic day 11.5 (E11.5) to form deep layer (Layer VI) in neocortex. As the development continue, the new neuroblasts migrate through and form a new layer above the existing layer. Prenatal neurogenesis continues until all layers are formed. Deep layer neurons, in particular, from various regions of neocortex start extending axons into respective thalamic region.
BIG1, a brefeldin A-inhibited guanine nucleotide-exchange protein, coded by Arfgef1 gene, have a Sec7 domain that activates Arf1 and Arf3 by replacing bound GDP with bound GTP, and thus, involves in vesicular trafficking in various cell types. In addition, BIG1 has also been found to regulate neurite outgrowth and maturation in vitro.
Using a BIG1 knockout ($BIG1^{-/-}$) mice model, the role of BIG1 in neocortex development and axonal connectivity was examined. Indeed, BIG1 knockout caused morphological defects in neocortex and pallial/subpallial boundary (PSPB). In BIG1 knockout neocortex, apoptosis occurred in early-born deep layer neurons and reached a peak soon after deep layer neuron differentiation at E15.5. Immunofluorescense staining using axonal or dendritic marker showed that the neuronal connectivity defects were observable only after E15.5. At E17.5, in BIG1

knockout brain, the corticothalamic axons from primary somatosensory cortex (S1) extended into dorsal lateral geniculate nucleus (dLGN) instead of ventrobasal complex (VB). The thalamocortical axons were unable to cross internal capsule (IC). Primary hippocampus neuron culture at 9 days-in-vitro (DIV) showed abnormal accumulation of NCKX2, a calcium channel protein that is a cargo for axonal motor protein Kif21a, at plasma membrane of dendrites. This might suggest that the loss of BIG1 lead to abnormal trafficking of NCKX2 through Kif21a, whose cargo binding site can also bind to BIG1 in wild type neurons.
This study showed the role of BIG1 in maintaining deep layer neuron survival and subsequent neuronal connectivity in developing embryonic brain. BIG1 may also function as a mediator for selective NCKX2 distribution at axon surface.

Disclosures: **J. Teoh:** None. **T. Iwano:** None. **M. Kunii:** None. **A. Harada:** None.

Poster

676. Cytoskeletal Mechanisms Underlying Axon Outgrowth and Guidance

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 676.17/C21

Topic: A.05. Axon and Dendrite Development

Title: Actin/spectrin periodicity at synapses and nodes of Ranvier reveald by STED microscopy

Authors: ***E. DESTE**[1], D. KAMIN[1], S. SIDENSTEIN[1], C. VELTE[2], M. SIMONS[2], S. HELL[1]; [1]Max Planck Inst. For Biophysical Chem., Goettingen, Germany; [2]Max Planck Inst. of Exptl. Med., Goettingen, Germany

Abstract: A ~190 nm periodic subcortical cytoskeleton lattice consisting of actin, spectrin, and other proteins was recently discovered underneath the membrane of hippocampal neurons and at nodes of Ranvier. By performing three-color STED nanoscopy, we show that this lattice is present in thick spine necks but is absent from post-synaptic sites, identified by Homer co-staining. Similarly, the lattice is discontinued at pre-synaptic sites, identified by Bassoon co-staining.
Whether the periodic cytoskeleton lattice is a structural feature of all neurons and how it is modified when axons are ensheathed by myelin-forming glial cells is not known. Therefore, we first confirmed the presence of the actin/spectrin lattice in different excitatory and inhibitory neuron types from both the central nervous system (cortical neurons, striatal neurons, cerebellar granule cells, retinal bipolar cells) and the peripheral nervous system (dissociated dorsal root ganglion cells). The lattice was also revealed in myelinating cells, specifically in cultured oligodendrocyte precursors, indicating that it is not a unique neuronal feature. The actin/spectrin lattice arrangement was found underneath the myelin coat at both internodes and paranodes of

sciatic nerve fibers *ex-vivo*. Co-staining of betaII and betaIV spectrin shows a continuity of this structure between paranodes and nodes, without any interruption. Consistent with the finding on oligodendrocyte precursor cells, at paranodes also glial cells exhibit a periodic arrangement of cytoskeletal and related proteins (Ankyrin B and Neurofascin 155).
In conclusion, we show that the subcortical actin/spectrin lattice is ubiquitous throughout the nervous system, being present in a variety of neuronal cell types. Our results suggest the existence of mechanisms that allow a fine-tuning of this substructure and open up many questions regarding both synaptic plasticity and de-myelination diseases.

Disclosures: **E. Deste:** None. **D. Kamin:** None. **S. Sidenstein:** None. **C. Velte:** None. **M. Simons:** None. **S. Hell:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); SWH owns shares of the companies Abberior GmbH and Abberior Instruments GmbH, producing dyes and microscopes for superresolution microscopy, respectively..

Poster

676. Cytoskeletal Mechanisms Underlying Axon Outgrowth and Guidance

Location: Halls B-H

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Topic: A.05. Axon and Dendrite Development

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Human Frontiers Science Program Long-term Fellowship (S.K.K.)

Title: Molecular mechanisms underlying polarized mitochondria structure and function in cortical neurons

Authors: *T. LEWIS, JR[1], S.-K. KWON[1], R. SHAW[2], F. POLLEUX[1];
[1]Dept. of Neurosci., Columbia Univ. Med. Ctr., New York, NY; [2]Salk Inst. for Biol. Studies, La Jolla, CA

Abstract: The importance of mitochondria for neuronal function is evident by the large number of neurodegenerative diseases (ALS, Alzheimer's disease, Parkinson's disease, Friedreich ataxia) which have been associated with a disruption of mitochondrial function or transport (Chen & Chan, 2009). Mitochondria are essential for multiple physiological functions as a result of their

ability to produce ATP through oxidative phosphorylation, buffer cytoplasmic calcium, regulate lipid biosynthesis and trigger apoptosis through cytochrome c release (Schon & Przedborski, 2011). In non-neuronal cells, mitochondria are highly dynamic as a result of mitochondrial biogenesis, fission, fusion, transport and removal via mitophagy. In neurons, mitochondria structure differs strikingly between the axon and dendrites: in axons, mitochondria are small (on average ~1 microns length) and often localized presynaptically whereas in dendrites, they form long, tubular networks covering almost the entire dendritic arbor. Therefore, we hypothesized that mitochondrial fission must be dominant over fusion in axons and vice versa in dendrites. These observations suggest highly polarized molecular mechanisms controlling mitochondria dynamics and function in axons vs. dendrites, however, at this point, the molecular mechanisms underlying compartmentalized mitochondrial fission/fusion in neurons are currently unknown. Recently, we have identified that Mitochondrial Fission Factor (MFF) activation (one of four known Drp1 'receptors'), controls mitochondrial size in both cell lines and neuronal dendrites in an AMPK-dependent manner (Toyama, Herzig et al, *Science* 2016). We will present results demonstrating that mitochondrial fission is regulated differentially by MFF in axons compared to the somatodendritic domain. We provide evidence that MFF-dependent mitochondrial fission is dominant in the axon, and that knockdown of MFF (but not Fis1) results in elongated, fused mitochondria along the axon without affecting dendritic mitochondrial morphology. Finally, disruption of mitochondrial fission in the axon results in changes to mitochondrial presynaptic localization resulting in changes to axonal morphology and mitochondrial-dependent calcium buffering function.

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Poster

676. Cytoskeletal Mechanisms Underlying Axon Outgrowth and Guidance

Location: Halls B-H

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Program#/Poster#: 676.19/C23

Topic: A.05. Axon and Dendrite Development

Support: R01 GM085115

Title: Bilaterian giant ankyrins have a common evolutionary origin and a conserved role in axon initial segment organization

Authors: ***T. J. JEGLA**[1], M. M. NGUYEN[2], D. J. GOETSCHIUS[2], E. LUNA[2], D. B. VAN ROSSUM[2], B. KAMEL[2], A. PISUPATI[2], E. S. MILNER[2], M. M. ROLLS[2];
[1]Biol., Pennsylvania State Univ. Dept. of Biol., University Park, PA; [2]Penn State, University Park, PA

Abstract: In vertebrate neurons, the axon initial segment (AIS) is specialized for action potential initiation. It is organized by a giant 480 Kd variant of ankyrin G (AnkG) that serves as an anchor for ion channels and is required for a plasma membrane diffusion barrier that excludes somatodendritic proteins from the axon. An unusually long exon required to encode this 480Kd variant is thought to have been inserted only recently during vertebrate evolution, so the giant ankyrin-based AIS scaffold has been viewed as a vertebrate adaptation for fast, precise signaling. We re-examined AIS evolution through phylogenomic analysis of ankyrins and by testing the role of ankyrins in proximal axon organization in a model multipolar *Drosophila* neuron (ddaE). We find giant isoforms of ankyrin in all major bilaterian phyla, and present evidence in favor of a single common origin for giant ankyrins and the corresponding long exon in a bilaterian ancestor. This finding raises the question of whether giant ankyrin isoforms play a conserved role in AIS organization throughout the Bilateria. We examined this possibility by looking for conserved ankyrin-dependent AIS features in *Drosophila* ddaE neurons via live imaging. We found that ddaE neurons have a diffusion barrier in the proximal axon that requires a giant isoform of the neuronal ankyrin Ank2. Our results indicate that the giant ankyrin-based cytoskeleton of the AIS may have evolved prior to the radiation of extant bilaterian lineages, much earlier than previously thought.

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Poster

676. Cytoskeletal Mechanisms Underlying Axon Outgrowth and Guidance

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Topic: A.05. Axon and Dendrite Development

Support: Préstamo BID PICT2010-1277

Préstamo BID PICT2014-3729

ISN-CAEN Award 2014-1C

Title: Characterizing cytoskeleton changes during axonal degeneration

Authors: ***N. UNSAIN**[1], M. BORDENAVE[2], S. JALIL[1], J. I. MARÍN[1], C. VON BILDERLING[2], A. D. JOHNSTONE[3], M. BISBAL[1], P. A. BARKER[3], F. D. STEFANI[2], A. O. CÁCERES[1];

[1]Inst. Ferreyra - INIMEC-CONICET-UNC, Córdoba, Argentina; [2]Ctr. de Investigaciones en Bionanociencias (CIBION), Buenos Aires, Argentina; [3]Vice Principal Research, Univ. of British Columbia Okanagan campus, Kelowna, BC, Canada

Abstract: Axonal fragmentation is a regulated process that uses a growing set of receptors, signaling molecules, proteases and other regulators to disintegrate the axonal compartment. Little is known about the changes (and possible role) of the axonal cytoskeleton during degeneration. In this study we aimed at describing cytoskeletal changes associated with axonal degeneration induced by of trophic factor (NGF) withdrawal (TFW) or injury in cultured DRG neurons. We first focused our attention in the actin-rich growth cone and found that growth cone collapse (GCC) is an early event observed in TFW and injured axons. Live-imaging shows that GCC is almost complete by 30 minutes after TFW, which is well before the 18-24 hours needed to observe axon fragmentation. Accompanying increased filopodia dynamics at the axonal tip suggest that GCC is caused by a sudden increase in F-actin dynamics. To our surprise, drugs (Nicotinamide Adenine Dinucleotide, EGTA, go6976, N-Acetyl Cysteine) or gene deletions (Caspase-3 -/-) known to prevent axonal fragmentation do not prevent GCC. Growth cone collapse does not represent a point of no return towards axonal fragmentation since re-adding NGF after GCC (6-12 hours) prevents axonal fragmentation and growth cones are re-form. Total F-actin staining decreases in every cell compartment during degeneration. On the other hand, axonal microtubules form tight bundles and we observed an early and marked microtubule de-bundling in degenerating axons and microtubules curves at the tip of axons where growth cones collapsed. These previously unnoticed early changes in the cytoskeleton suggest an instructive role for the cytoskeleton during axon fragmentation.

Disclosures: **N. Unsain:** None. **M. Bordenave:** None. **S. Jalil:** None. **J.I. Marín:** None. **C. von Bilderling:** None. **A.D. Johnstone:** None. **M. Bisbal:** None. **P.A. Barker:** None. **F.D. Stefani:** None. **A.O. Cáceres:** None.

Poster

677. Neural Circuit Activity and Maturation II

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 677.01/C25

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Title: Weakening of GABAergic connections from somatostatin interneurons to pyramidal neurons after eye-opening in layer 2/3 of visual cortex

Authors: *W. GUAN, J.-W. CAO, Y. FU, Y.-C. YU;
Inst. of Brain Sci., Fudan Univ., Shanghai City, China

Abstract: A precise cortical GABAergic network is critical for the normal sensory coding and perception. However, how cortical GABAergic circuits are developmentally assembled and regulated in the early postnatal period is far from fully understood. Eye opening is a timed event critical for the maturation of cortical circuits, yet our knowledge of the relationship between eye-opening and the maturation of specific GABAergic connections is incomplete. Here we report first time that, 1-2 days after eye-opening, the IPSC amplitude from somatostatin interneurons to pyramidal neurons abruptly decrease by about 50% in layer 2/3 of the visual cortex. Similar results were not found in connections from somatstatin interneurons to other interneurons subtypes. What's more, the reduction of IPSC amplitude is impared by the disruption of normal visual input, suggesting that the developmental change after eye-opening is regulated by early visual experience. Our work revealed a novel early developmental pattern of GABAergic circuits around the time eye opens and indicated that early visual experience is critical for the maturation of somatostatin interneuons GABAergic circuits in visual cortex.

Disclosures: **W. Guan:** None. **J. Cao:** None. **Y. Fu:** None. **Y. Yu:** None.

Poster

677. Neural Circuit Activity and Maturation II

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 677.02/C26

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Title: Function of early generated interneurons in the developing neocortex of neonatal mouse

Authors: ***C. WANG**, S. JIANG, J. MA, L. ZHANG, Z. YUAN, X. MAO, Y. YU;
Inst. of Brain Sci., Fudan Univ., Shanghai, China

Abstract: The generation of neocortex interneurons approximately starts from E9.5 and ends at E18.5. Previous studies have revealed that a portion of early generated interneurons (EGIns) in the hippocampus can act as hub neurons which may modulate the synchronized activity of the hippocampus network. However, the functional significance of EGIns in neocortex is not well understood. In this study, combining fast multi-scale calcium imaging (fMCI) with whole cell recording, we found that some EGIns in the neocortex also have hub function. On the basis of electrophysiological and morphological data, we observed that EGIns have a higher connectivity level than ordinary generated interneurons (OGIns) which were born around the peak of neurogenesis. Nevertheless, this advantage of EGIns almost only occurs in the first postnatal week and disappears later when the neocortex gets more mature. Our results implied that EGIns have a stronger regulatory function in the developing neocortex at early postnatal stage when vast cortical synaptic network begin to establish.

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Poster

677. Neural Circuit Activity and Maturation II

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Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: CIHR Doctoral Vanier CGS

CIHR Foundation Grant Edward Ruthazer

Title: Distinct roles for presynaptic and postsynaptic nmdars in visual circuit development

Authors: ***P. M. KESNER**, E. C. WARREN, F. MA, E. S. RUTHAZER;
Neurol. and Neurosurg., Montreal Neurolog. Inst., Montreal, QC, Canada

Abstract: The N-methyl-D-aspartate type glutamate receptor (NMDAR) has been strongly implicated as playing a role in retinotectal projection refinement, arbor stabilization, and plasticity during development. Chronic receptor blockade experiments have demonstrated that NMDARs in the optic tectum play a key role in retinotectal axon remodeling and refinement. Once thought to be exclusively postsynaptic, more recent work has revealed the existence of presynaptic NMDARs (preNMDARs) that may also contribute to synaptic function and plasticity. We have therefore undertaken a set of experiments to specifically assess the relative contributions to synaptic plasticity and circuit refinement of pre- versus postsynaptic NMDARs (postNMDARs). To do so, we have developed a novel model system in which we inject an antisense Morpholino oligonucleotide (MO) against the obligate GluN1 subunit of the NMDAR into just one cell at the two-cell stage of development. This results in the NMDAR being effectively knocked-down in half the embryo. Because the retinal ganglion cells (RGC) cross the midline to project to the optic tectum, these hemimorphant animals lack NMDARs in all the RGC inputs to their wildtype contralateral hemisphere and in all the tectal neurons (with wildtype RGC inputs) in the other hemisphere. Preliminary findings suggest complementary contributions to axon complexity and dynamics for pre- and postNMDARs as well as alterations in visual response properties. These early findings set the groundwork for a more comprehensive investigation of the specific roles of pre- and postsynaptic NMDARs in neural circuit development, plasticity, and visual processing.

Disclosures: **P.M. Kesner:** None. **E.C. Warren:** None. **F. Ma:** None. **E.S. Ruthazer:** None.

Poster

677. Neural Circuit Activity and Maturation II

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Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: The Ministry of Education, Culture, Sports, Science and Technology of Japan

Opto-Medical Institute

Title: Optical imaging of widely-spreading wave activity in the embryonic chick forebrain induced by olfactory nerve stimulation

Authors: *K. SATO[1], Y. MOMOSE-SATO[2];
[1]Dept. of Hlth. and Nutr. Sci., Komazawa Women's Univ, Fac. of Human Hlth., Tokyo, Japan;
[2]Dept. of Nutr. and Dietetics, Kanto Gakuin Univ., Col. of Nutr., Yokohama, Japan

Abstract: We have applied multiple-site optical recording with a voltage-sensitive dye to the olfactory nerve (N.I)-olfactory bulb-forebrain preparation of the chick embryo, and pursued functional development of the olfactory system. In our previous studies, we showed that optical responses in the olfactory bulb induced by N.I stimulation consisted of three components, viz., a fast spike-like signal, a delayed long-lasting slow signal, and an oscillation. The fast spike-like signal corresponded to the sodium-dependent action potential, and the slow signal included the glutamatergic excitatory postsynaptic potential. Functional synaptic transmission in the olfactory bulb was expressed at the embryonic 6-7-day stage, while the oscillation was detected from the embryonic 9-day stage. In the present study, we found that the slow signal spread into the forebrain as a wave-like activity and distributed widely in the cortex in some conditions. This wave-like activity was elicited from the embryonic 9-day stage in normal physiological solution and the 8-day stage in the Mg^{2+}-free solution. We examined fundamental characteristics of the wave-like activity and their developmental dynamics.

Disclosures: K. Sato: None. **Y. Momose-Sato:** None.

Poster

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KAKENHI 15H01454

KAKENHI 15H04263

KAKENHI 16K14559

Title: *In vivo* 2-photon imaging of neuronal activity in layer 4 of the neonatal barrel cortex

Authors: *H. MIZUNO[1,2], T. SATO[1], T. IWASATO[1,2];
[1]Natl. Inst. of Genet., Mishima / Shizuoka, Japan; [2]Genet., SOKENDAI, Mishima / Shizuoka, Japan

Abstract: Establishment of precise neuronal circuits in the mammalian neocortex relies on activity-dependent circuit reorganization during postnatal development; however, the nature of cortical activity that could underlie the circuit refinement remains largely unknown. We addressed this question using the mouse somatosensory cortex. In the mouse somatosensory cortex layer 4, the arrangement of whiskers on the face is topographically represented as "barrels", which are discrete modules of layer 4 neurons. Thalamocortical (TC) axon termini that correspond to a whisker are clustered in the center of a single barrel, and dendrites of a layer 4 neuron are mostly restricted to a single barrel. These highly organized TC circuits are established during neonatal stages, in which neuronal activity via thalamus plays a critical role. We first developed a method to analyze activity of layer 4 neurons with respect to the TC axon projection patterns: To observe the arrangement of barrels (TC axon terminus clusters) in vivo, we generated transgenic mice expressing RFP in TC axons (TCA-RFP mice). We expressed genetically encoded calcium indicator GCaMP in a sparse population of layer 4 neurons in these mice. Sparse cell labeling was achieved by using the Supernova system, which we recently reported (Mizuno et al., Neuron 2014). We then monitored neuronal activity in the somatosensory cortical layer 4 during neonatal stages, and analyzed features of the activity with

respect to the TC input patterns. The properties of cortical activity during early postnatal period that may be involved in circuit refinement will be discussed.

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Poster

677. Neural Circuit Activity and Maturation II

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Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: Research to Prevent Blindness Walt and Lilly Disney Award for Amblyopia Research

NIH T32EY007120

Title: Antagonistic roles of serotonin and glutamate release in the refinement of retinogeniculate circuits

Authors: ***D. Q. DAO**[1], B. GIROS[2], E. M. ULLIAN[1];
[1]Ophthalmology, Univ. of California San Francisco, San Francisco, CA; [2]Psychiatry, Douglas Mental Hlth. Res. Center, McGill Univ., Montreal, QC, Canada

Abstract: During early postnatal development in mouse retinorecipient areas, retinal projections are broad and diffuse, exemplified by the highly overlapping eye-specific (ipsi- vs. contralateral) retinal projections in the dorsal lateral geniculate nucleus (dLGN). A Hebbian mechanism was hypothesized to orchestrate the large-scale refinement of these eye-specific circuits, resulting in a highly segregated organization of eye-specific connectivity. Previously, we explored the role of glutamate release in the refinement of eye-specific inputs to the dLGN, concluding that glutamate release from ipsilateral fibers is important for the removal of contralateral fibers in the territory destined to be ipsilateral, whereas the maintenance and refinement of ipsilateral fibers does not require normal levels of glutamate release. We sought to uncover the molecular signal that conveys this latter aspect of retinogeniculate refinement through examination of serotonin release. The model of serotonin's role in refinement of the retinogeniculate synapse had long been thought to be inhibitory onto contralateral inputs via release from ipsilateral inputs. However, using conditional genetic deletion of serotonin's vesicular transporter (VMAT2) in ipsilaterally-projecting retinal ganglion cells (ipsi-RGCs), we found that loss of serotonin release from ipsi-RGC unexpectedly accelerates refinement leading to early and complete refinement of inputs. Furthermore, genetic disruptions of both glutamate and serotonin release surprisingly rescued the retinogeniculate refinement phenotype observed after disrupted glutamate or

sertotonin release alone. These results suggest an antagonistic role of glutamate and serotonin release from ipsi-RGCs and furthermore demonstrate that normal developmental circuit refinement is capable after reduction of both transmitters. These results update our current understanding of the role of serotonin and glutamate signaling over neural circuit refinement during development.

Disclosures: **D.Q. Dao:** None. **B. Giros:** None. **E.M. Ullian:** None.

Poster

677. Neural Circuit Activity and Maturation II

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KAKENHI 15H04263

Title: Supernova: Extensible vector systems that enable high intensity single-cell labeling and labeled cell-specific gene knockout and editing *In vivo*

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

Abstract: The mammalian brain, a complex organ, comprises numerous cells (neurons) densely packed and interconnected with each other to form intricate neural circuits responsible for higher brain function. To understand the precise cellular and molecular mechanisms of the neural circuit development and function, single-cell analyses that dissect connectivity of individual cells and molecular machinery operating in these cells are indispensable. For this purpose, we developed “Supernova” series of vector systems. In Supernova, sparse labeling relies on rare TRE leakage.

In a small population of cells with over-threshold leakage, initial tTA-independent weak expression is enhanced by tTA/TRE-positive feedback along with a site-specific recombination system (e.g., Cre/loxP, Flpe/FRT). Sparse and bright labeling by Supernova with little background enables the visualization of the morphological details of individual neurons in densely packed brain areas such as the cortex and hippocampus, both during development and in adulthood. Sparseness levels are adjustable. Labeled cell-specific gene knockout was accomplished by introducing Cre/loxP-based Supernova vectors into floxed mice. Furthermore, by combining with RNAi, TALEN, and CRISPR/Cas9 technologies, Supernova achieved labeled cell-specific gene knockdown and editing/knockout without requiring genetically altered mice. Thus, Supernova system is highly extensible and widely applicable for single-cell analyses in complex organs, such as the mammalian brain.

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Poster

677. Neural Circuit Activity and Maturation II

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Swiss National Science Foundation, Advanced Post-Doc mobility fellowship (P300PB_164753)

Title: Layer specific developmental changes of excitatory and inhibitory balance in pyramidal neurons of rat primary visual cortex

Authors: *R. TATTI, A. MAFFEI;
Stony Brook Univ., Stony Brook, NY

Abstract: Neural circuits are highly sensitive to changes in environmental inputs, especially early in life when windows of heightened plasticity have been reported. These developmental windows are known as “critical periods”. In rat primary visual cortex (V1) the best studied of the critical periods was shown to open at postnatal day 21 (P21) and peak by the end of the fourth week. In addition, a second window of plasticity was also identified. It is known as pre-critical period, starts at eye opening and extends to P21. In these two developmental windows the circuit

in V1 appears to have different sensitivity to patterned stimulation. For example, the same pattern of activity can induce completely different forms of plasticity if applied in the pre-critical or in the critical period, suggesting that the local circuit may be in different states of excitability. Here, we recorded spontaneous excitatory and inhibitory currents from pyramidal neurons (Pyr) in different layers of V1 at eye opening and the peak of the critical period. Our data show that there are laminar differences in the balance between excitation and inhibition (E/I) in V1 Pyr neurons recorded at these two developmental stages. At eye opening (P14) spontaneous activity was dominated by excitation in layer L4 (E/I ratio in L4 =1.29 ± 0.14, *n*=12 Pyr neurons) while inhibition was dominant in L2/3, L5 and L6 (E/I ratios: L2/3=0.39 ± 0.06, L5=0.72±0.2, L6=0.81±0.1 respectively, *n*=12 neurons for each layer). At the peak of the critical period inhibition was dominant throughout the cortical mantle (L2/3=0.1±0.02, L4=0.2±0.02, L5=0.2±0.02, L6=0.1±0.01). Our results indicate that from eye opening and the peak of the critical period there is a progressive shift toward inhibition becoming dominant in all cortical layers. This transition is likely to contribute to the maturation of the response properties and to the changes in capacity for plasticity of neurons in V1.

Disclosures: **R. Tatti:** None. **A. Maffei:** None.

Poster

677. Neural Circuit Activity and Maturation II

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 677.09/C33

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Support: NIH Grant 532NS084749

NIH Grant 5F32NS071807

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Hahn Family Foundation

Title: Convergent tectal inputs drive circuit plasticity and visuomotor behavior via changes in excitatory to inhibitory balance

Authors: *A. C. GAMBRILL[1], R. L. FAULKNER[2], H. CLINE[2];
[1]The Scripps Res. Inst., La Jolla, CA; [2]The Scripps Res. Inst., San Diego, CA

Abstract: Communication between tecta/colliculi is thought to be required for sensorimotor behaviors by comparing input across the midline, however the role of visual experience in the

function and plasticity of developing intertectal (IT) connections in unclear. Using in vivo time-lapse imaging, electrophysiology, and visuomotor behavior experiments in *Xenopus* tadpoles, we examine the experience-dependent plasticity and function of intertectal inputs. We find that intertectal neurons provide convergent excitatory and inhibitory input onto retinorecipient tectal neurons, and that these intertectal inputs are required for visuomotor behavior. We show that spike-timing dependent plasticity of intertectal inputs and experience-dependent structural plasticity in presynaptic excitatory and inhibitory IT boutons change the balance of excitation to inhibition (E:I) of intertectal input to tectal neurons. Intertectal inputs are required for visual avoidance behavior and exposing tadpoles to unilateral patterned visual input impairs behavior and disrupts E:I balance. These results demonstrate that visual experience induces plasticity in IT synapses and that discordant retinal inputs shift the E:I ratio of IT input onto tectal neurons, which is sufficient to transiently disrupt the integration of visual information and motor output in the tectal circuit and to disrupt visuomotor behavior. This work was supported by NIH grants 532NS084749 to ACG, 5F32NS071807 to RLF, and EY011261 to HTC and an endowment from the Hahn Family Foundation to HTC.

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Poster

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Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: Grant from DBT, India

Funding from NCBS, TIFR

Title: Store-operated calcium entry in pupal *Drosophila* neurons regulates flight through Ral expression and vesicular release

Authors: ***S. RICHHARIYA**[1], S. JAYAKUMAR[1,2], K. ABRUZZI[3], M. ROSBASH[4], G. HASAN[1];
[1]NCBS, TIFR, Bangalore, India; [2]Manipal Univ., Manipal, India; [3]Brandeis Univ., Waltham, MA; [4]HHMI, Brandeis Univ., Waltham, MA

Abstract: Transcriptional regulation by Store-operated Calcium Entry (SOCE) is well studied in non-excitable cells. However, the role of SOCE has been poorly documented in neuronal cells with heterogeneous calcium dynamics. We study the role of SOCE in the developing nervous

system of *Drosophila*. Previous reports demonstrated a requirement for SOCE in *Drosophila* flight circuit neurons. We refine this requirement temporally to the early pupal stage and use RNA sequencing to identify SOCE mediated gene expression changes in the developing *Drosophila* pupal nervous system. Down regulation of *dStim*, the endoplasmic reticular calcium sensor and a principal component of SOCE in the nervous system, altered the expression of 131 genes including *Ral*, a small GTPase. Disruption of Ral function in neurons impaired flight, whereas ectopic expression of *Ral* in SOCE compromised neurons restored flight. We demonstrate, by live imaging of cultured pupal neurons, a role for SOCE dependent *Ral* expression in regulating vesicular exocytosis. Compromising vesicle release in early pupal neurons alone, hampers flight, supporting the functional significance of *Ral* expression at this developmental stage. These results identify neuronal SOCE as a mechanism that regulates *Ral* expression and consequently neural function and behaviour. They also demonstrate the relevance of SOCE regulated gene expression for neuronal circuit maturation.We also profiled the pupal transcriptome of Dopaminergic and Glutamatergic, two known neuronal domains where SOCE has been implicated in flight, under conditions of impaired dSTIM function to study the role of SOCE in these neurons. Interestingly, we find SOCE to differentially regulate a set of genes in these two subsets.

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Poster

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Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: 5R01NS086932-02

Title: Role of early activity in the development of the *Caenorhabditis elegans* egg laying behavior circuit

Authors: *B. RAVI, K. M. COLLINS;
Biol., Univ. of Miami, Miami, FL

Abstract: Early activity has been shown to be important for the proper development and assembly of neural circuits. Activity is also required to form coordinated patterns in mature neural circuits that drive efficient behaviors. We are interested in studying the functional importance of early activity using the developing egg-laying behavior circuit in the nematode

Caenorhabditis elegans. The *C. elegans* egg laying circuit is a simple, well characterized neural circuit which drives a two-state behavior in which adult animals alternate between an inactive state and an active state when eggs are laid. The circuit comprises two types of motor neurons: the Hermaphrodite Specific Neurons (HSNs) and Ventral Type C motor neurons (VC) that innervate the vulval muscles which contact to lay eggs. During the L4 larval stage, the cells of the circuit undergo morphological maturation and establish synaptic connections. We are using GCaMP calcium imaging in freely behaving animals to record activity in cells of the egg laying circuit at the late L4 larval stage and in adults after development. We found the HSN neurons have rhythmic Ca^{2+} transients at the late L4 larval stage at intervals of about 55s even before eggs are produced. HSN Ca^{2+} transients are also observed at the mid-L4 stage, but are less frequent than during late-L4 (1hr post mid-L4). HSN activity in late-L4 animals lacked the characteristic burst-firing pattern at intervals of about 20s in adults during egg laying. Developing vulval muscles have Ca^{2+} activity during the transition from mid-L4 to late-L4 and after the L4 molt is complete. Vulval muscle L4 Ca^{2+} transients are uncoordinated at the mid-L4 stage, where we see asynchronous Ca^{2+} transients at either the anterior or posterior muscle cells. At the end of the L4 stage, coordinated muscle transients appear, which resembles the activity seen in adult muscles that allows efficient egg release. In order to understand the functional consequences of circuit connectivity, we are analyzing how the observed activity correlates with morphological changes observed during circuit development. We are also analyzing the role of early activity in regulating other behaviors that change during the L4 stage, including locomotion and pharyngeal pumping. We hope to test how acute and/or developmental perturbations in cell activity drive functional and behavioral plasticity. Together, these results will help us to understand how normal patterns of cell activity allow for proper development of neural circuits that drive robust, stable behaviors.

Disclosures: B. Ravi: None. **K.M. Collins:** None.

Poster

677. Neural Circuit Activity and Maturation II

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Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: The Australian Research Council, Discovery Grant DP150101152

Title: Development of spontaneous activity in the larval zebrafish tectum

Authors: *L. AVITAN[1], Z. PUJIC[1], B. SUN[1], E. K. SCOTT[2], G. J. GOODHILL[1,3];
[1]Queensland Brain Inst., The Univ. of Queensland, Brisbane, Australia; [2]The Univ. of

Queensland,, Sch. of Biomed. Sci., Brisbane, Australia; [3]The Univ. of Queensland, Sch. of Mathematics and Physics, Brisbane, Australia

Abstract: Spontaneous patterns of activity in the developing visual system may play an important role in shaping the brain to optimally reflect the statistics of the visual environment. During the period 4-9 dpf (days post-fertilization) larval zebrafish learn to hunt prey, a behaviour critically dependent on the optic tectum. However whether and how spontaneous activity in the tectum evolves over this period is unknown. To address this we performed 2-photon calcium imaging of GCaMP6s unanesthetised, unparalysed zebrafish larvae in the dark for 1 hour at ages 4-9 dpf. We recorded spontaneous activity of ~600 active tectal cells per age (n = 48 fish, an average of 8 fish per age), and found significant changes in activity characteristics over development. From 4 to 5 dpf the frequency of spontaneous activity events in the PVL (periventricular layer) increased, and remained elevated until 8 dpf, when it returned to the same level as 4 dpf. Cells within the neuropil (n=410 total over all ages) segregated both functionally and spatially into two groups with different spontaneous activity profiles. PVL activity took the form of structured assemblies, whose spatial and temporal characteristics also changed over development. Preliminary results indicate a strong role for retinal input in shaping this tectal spontaneous activity, since fish with one eye enucleated showed substantially reduced activity in the enucleated vs normal tectum. Together these findings show that spontaneous activity characteristics in the zebrafish tectum change rapidly over the period in which the animal is learning to process visual stimuli and hunt prey, and that visual experience may play a role in driving these changes.

Disclosures: L. Avitan: None. **Z. Pujic:** None. **B. Sun:** None. **E.K. Scott:** None. **G.J. Goodhill:** None.

Poster

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Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: NSERC

Fonds de recherche en santé du Québec

Title: Role of histone deacetylase 2 (HDAC2) in PV cell circuit development

Authors: *M. LAVERTU JOLIN, T. BADRA, G. DI CRISTO;
CHU Sainte-Justine / Univ. De Montréal, Montreal, QC, Canada

Abstract: Cortical parvalbumin-positive, basket cells (PV cells), a subtype of GABAergic interneurons, innervate hundreds of postsynaptic targets with multiple synapses clustered around the cell body and proximal dendrites. These cells are thought to be particularly important for the generation of gamma oscillations, which in turn regulate many cognitive functions, and for the regulation of developmental cortical plasticity. Although the function of PV cells within cortical networks is being explored extensively, the mechanisms that control the development and plasticity of their extensive arborization and synaptic contacts have not been entirely resolved. Molecular mechanisms involved in synapse formation and strengthening include the activation and repression of specific genes or subsets of genes by stable epigenetic modifications that do not change the genetic code itself. Chromatin remodeling, especially through histone-tail acetylation, which alters the compact chromatin structure and changes the accessibility of DNA to regulatory proteins, is emerging as a fundamental mechanism for regulating gene expression. In particular, Histones Deacetylase 2 (HDAC2) has been shown to regulate excitatory synapse formation and plasticity, and memory formation. Whether HDAC2 affects PV cell synapse development is unknown. Here, we show that HDAC2 is expressed by PV neurons. To dissect the role of HDAC2 in PV cell development *in vivo*, we generated conditional knockout mice by breeding $HDAC2^{lox}$ with PV-Cre mice, which express Cre selectively in PV cells after the second postnatal week. We found that PV expression levels and PV cell perisomatic boutons density is significantly reduced in both the cortex and basal lateral amygdala by P60. We are currently characterizing the cognitive behavior of these mice.

Disclosures: **M. Lavertu Jolin:** None. **T. Badra:** None. **G. Di Cristo:** None.

Poster

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Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: JST PRESTO

JSPS Kakenhi 23680038

JSPS Kakenhi 15K14336

JSPS Kakenhi 15K14327

Title: Intrinsic spontaneous network activity in the neonatal mouse olfactory bulb is required for dendrite pruning of mitral cells.

Authors: *M. N. LEIWE[1], S. FUJIMOTO[1], Y. MUROYAMA[2], R. KOBAYAKAWA[3], K. KOBAYAKAWA[3], T. SAITO[2], T. IMAI[1];
[1]Ctr. for Developmental Biol., RIKEN, Kobe, Japan; [2]Dept. of Developmental Biol., Chiba Univ., Chiba, Japan; [3]Inst. of Biomed. Sci., Kansai Med. Univ., Osaka, Japan

Abstract: The correct formation of neural circuits is vital for the function of the brain, and is achieved by both the correct outgrowth and pruning of neurites. In the mouse olfactory bulb (OB), mitral and tufted cells (M/T cells) are the principal output neurons, receiving input from olfactory sensory neurons (OSNs) in the glomerular layer of the OB, and projecting axons to the olfactory cortex. During postnatal development, M/T cells refine their dendrites from connecting to multiple glomeruli (Postnatal days 0-3/4) to a single primary dendrite connecting to a single glomerulus ≥P5). However, the mechanisms of this selective pruning remain unclear.
Our genetic experiments uncovered a critical role for non-sensory (spontaneous) activity in shaping this refinement. Notably, hyperpolarising mitral cells through the expression of the inward rectifying potassium channel Kir2.1 caused mitral cells to retain multiple primary dendrites well beyond the typical pruning period (>P28). Naris occlusion or altering odour sensitivity genetically resulted in no or only a slight delay in refinement.
Although the role of spontaneous activity in circuit refinement is established in visual and auditory circuit development, the nature of spontaneous activity in the olfactory system is unknown. To directly record spontaneous activity in the neonatal mouse OB, we performed two-photon *in vivo* imaging with genetically encoded calcium indicators (GCaMP3/6f) expressed either in M/T cells or OSNs across the early postnatal development (≤P10). Spontaneous activity was present in M/T cells in awake mice, but not in OSNs, suggesting that it is intrinsic to the OB, consistent with our genetic experiments. We also found a significant shift from synchronous to asynchronous firing during postnatal development. Using correlation analyses, we demonstrated synchronous firing at earlier stages (P0-2, stage I), and desynchronized and seemingly random firing at later stages (stage II). Pharmacological experiments on ex-vivo explants revealed that synchronicity is dependent on both synaptic and gap junction connectivity for stage I spontaneous activity, while stage II activity requires GABAergic inhibition. We have also begun to analyse the activity patterns of mutant mice in which dendritic pruning is delayed.
This shift in spontaneous activity patterns occurs prior to dendritic pruning, suggesting that the stage II pattern of intrinsic OB spontaneous activity may be important to initiate the pruning of primary dendrites. Formation of discrete microcircuits by emergent network activity may be an important principle in the formation of mammalian central nervous system.

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Poster

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Simons Center for the Social Brain/SFARI

HFSP Long-Term Fellowship

Title: Major vault protein, a candidate gene in 16p11.2 microdeletion syndrome, is required for homeostatic regulation of cortical plasticity

Authors: *P. IP[1], I. NAGAKURA[1], J. PETRAVICZ[1], J. BENOIT[1], E. A. C. WIEMER[2], M. SUR[1];
[1]Picower Inst. for Learning and Memory, MIT, Cambridge, MA; [2]Inst. of Hematology, Erasmus Univ. Rotterdam, Rotterdam, Netherlands

Abstract: Microdeletion of a region in the chromosome 16p11.2 increases susceptibility to autism and accounts for up to 1% of this population. Although this region contains 29 genes, disrupting only a small piece of this region, which spans 5 genes, is sufficient to cause autistic traits. One candidate gene in this region is the major vault protein (MVP), which has been implicated in the regulation of several cellular processes including transport mechanisms and multidrug resistance. We found that MVP expression levels in MVP Het mice closely phenocopy those of 16p11.2 mice, suggesting MVP Het mice may serve as a model of MVP function in 16p11.2 microdeletion. However, the function of MVP in the central nervous system, in particular its role in brain function and plasticity, has not been investigated. To determine the role of MVP in experience-dependent synaptic and circuit plasticity, we first measured ocular dominance plasticity (ODP) in primary visual cortex (V1). We found that MVP Het mice show impairment in strengthening of open eye responses in V1 after 7 days monocular deprivation (MD), resulting in reduced overall plasticity. Furthermore, electrophysiology experiments showed that the frequency of mEPSCs was decreased in MVP Het mice after 7 days MD, suggesting a decrease in the number of functional synapses, which may underlie the reduced plasticity in MVP Het mice. By a biotin-labeling assay, we found impaired homeostatic upregulation of surface GluA1 in MVP Het mice after longer term MD. To investigate the underlying molecular mechanism, we measured intracellular signaling in MVP WT and MVP Het mice and found ERK activation was significantly increased in MVP MVP Het mice, while activation of other signals such as Akt and JAK were normal. STAT1 is a downstream molecule of JAK signaling and reported to be inhibited by MVP. We found a tendency towards increased

expression of STAT1 in MVP Het mice, suggesting the possibility of MVP inhibition on STAT1. We have previously examined ODP in STAT1 KO mice and shown that they have an accelerated increase in open eye responses and enhanced plasticity after 4 days of MD. These results suggest that MVP may interact with STAT1 to regulate plasticity, and one function of MVP may be to negatively regulate STAT1. Collectively, we find a highly specific role for MVP as a critical molecule in the homeostatic or response-restoring component of activity-dependent synaptic plasticity. Thus, this study helps reveal a new mechanism for an autism-related gene in brain function, and suggests a broader role for neuro-immune interactions in circuit level plasticity.

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Poster

677. Neural Circuit Activity and Maturation II

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Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: CREST

Title: High reciprocal connectivity between clonal cortical neurons is established under the guidance of epigenetic regulation

Authors: E. TARUSAWA[1,2], M. SANBO[1], A. OKAYAMA[3], T. MIYASHITA[1], T. KITSUKAWA[3], T. HIRAYAMA[3], T. HIRABAYASHI[3], S. HASEGAWA[3], M. HIRABAYASHI[1], T. YAGI[3,4], *Y. YOSHIMURA[1];
[1]Natl. Inst. For Physiological Sci., Okazaki, Aichi, Japan; [2]CREST /AMED, Tokyo, Japan; [3]Grad Sch. of Frontier Biosci, Osaka Univ., Osaka, Japan; [4]CREST/AMED, Tokyo, Japan

Abstract: The specificity of synaptic connections is fundamental for proper neural circuit function. Here we show that cortical excitatory neurons that arise from the same neural stem cell and reside within the same layer preferentially establish reciprocal synaptic connections, guided by molecular cues predetermined by epigenetic regulation during embryonic development. We observed a transient increase in synaptic connections between clonal but not nonclonal neuron pairs postnatally, followed by selective stabilization of the reciprocal connections between clonal neuron pairs. This lineage-dependent connective reciprocity was abolished in clonal cells lacking DNA methyltransferase 3b (Dnmt3b), which determines DNA-methylation patterns of genes in stem cells during early corticogenesis. A similar abolishment of reciprocity was observed in clonal neurons lacking clustered protocadherin (cPcdh), adhesion molecules involved in cell-cell

interactions. Dnmt3b regulated the postnatal expression of cPcdh isoforms. Our findings suggest that the Dnmt3b-mediated epigenetic regulation of cPcdh expression enables clonal neurons to establish cell-lineage-specific reciprocal connections.

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Poster

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Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: HKRGC17125115M & 777911M

Title: The regulatory roles of perineuronal semaphorin 3A and chondroitin sulfates on the developing vestibular circuitry

Authors: *D. K.-Y. SHUM[1,2], P. Y. KWAN[1], C. W. MA[1], Y. S. CHAN[1,2];
[1]Sch. of Biomedic. Sci., Fac. Med., Univ. Hong Kong, Hong Kong, China; [2]Res. Ctr. of Heart, Brain, Hormone and Healthy Aging, Fac. Med., Univ. Hong Kong, Hong Kong, China

Abstract: Perineuronal nets (PN) are crucial for restricting neuronal plasticity during development. Our study of the central vestibular nucleus (VN) found consolidation of PN around GABAergic interneurons as from postnatal day (P)9 of Sprague Dawley (SD) rats. This was accompanied by progressive localization of semaphorin 3A (Sema3A) to chondroitin sulphate moieties (CS) of PN. We hypothesized that PN-CS binding of Sema3A limits the action of Sema3A as a plasticity-inducing factor in the VN. We tested for structural plasticity in VN explant cultures (P3+up to 31DIV) following treatment with chondroitinase ABC (ChABC) and/or Sema3A. Parallel cultures were fixed for assessment of neurite arborization and growth. Compared with null treatment controls, increase in these parameters suggested involvement of CS and Sema3A in controlling structural plasticity of VN neurons. To study the impact of PN-CS/Sema3A at the circuit level, the rats were assessed for the emergence of negative geotaxis as a read-out for maturation of the circuit for graviception. We observed negative geotaxis as early as P9, in correlation with consolidation of PN around GABAergic neurons in the VN. ChABC/Sema3A-treated rats showed delayed display of negative geotaxis, similar to effects of bicuculline but contrasting those of muscimol. The delay also correlated with postponed formation of PN after ChABC treatment, revealing that disturbance of PN consolidation

interfered with maturation of the vestibular circuitry for graviception. We further performed whole-cell patch-clamp recordings of miniature excitatory post-synaptic current (mEPSC) from VN interneurons of P7/P9 rats, control versusthose treated with ChABC and Sema3A. We found increase in frequency of mEPSCs, suggesting strengthening of presynaptic signals along extended lengths of dendrites of interneurons in the ChABC/Sema3A-treated VN circuit. Our results provide evidence for the role of PN-CS-Sema3A in controlling structural and circuit plasticity at the interneuron level with impacts on the developmental display of graviceptive behaviour. [Grant Support HKRGC17125115M & 777911M]

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Poster

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Title: Differential effects of decreasing excitatory inputs on excitatory and inhibitory neurons

Authors: *H. HE[1], W. SHEN[2], H. CLINE[1];
[1]The Scripps Res. Inst., LA Jolla, CA; [2]Inst. of Developmental and Regenerative Biology, Col. of Life and Envrn. Sci., Hangzhou Normal Univ., Hangzhou, China

Abstract: AMPARs are the major receptors mediating fast excitatory synaptic transmission in the CNS. Trafficking of AMPARs to and from synapses plays pivotal roles in the excitatory neural transmission, thus affecting synaptic plasticity, structural plasticity, circuit function and behavior. Here, we studied the role of AMPAR-mediated fast excitatory synaptic transmission by expressing peptides corresponding to the C-terminus of GluA1 or GluA2 subunits (called GluA1 and GluA2 CTPs) in optic tectal neurons in Xenopus laevis tadpoles. In contrast to the effect of the disruption of inhibitory inputs, which significantly disrupts the E/I of visually-evoked synaptic inputs onto the postsynaptic neurons (Shen et al., 2011), GluA CTPs decreased both spontaneous and visually-evoked EPSC and IPSC onto tectal neurons. As a result, the E/I balance was unchanged. Nonetheless, the neuronal receptive field properties and the visually-guided avoidance behavior of the animals were all affected by the expression of GluA-CTPs. To

dissect the effect of disruption of excitatory inputs on excitatory versus inhibitory neurons respectively, we used time-lapse in vivo imaging to evaluate the effects of GluA CTP expression on dendritic arbor growth and experience-dependent structural plasticity in tectal neurons combined with posthoc GABA immunostaining to determine the identity (excitatory or inhibitory) of imaged neurons. The dendritic arbor structures of excitatory and inhibitory neurons were differentially affected by the disruption of excitatory inputs and demonstrated distinctive changes in experience-dependent structural plasticity as assayed by a two-part visual manipulation paradigm (4hrs of dark vs. 4hrs of enhanced visual stimulation). In particular, the excitatory neurons showed decreased dendritic branch density and mostly abolished their response to experience-potentiated dendritic arbor growth as results of expression of either GluA1-CTP or GluA2-CTP. On the other hand, inhibitory neurons did not exhibit significant change in the overall dendritic arbor morphometric parameters to expression of GluA1-CTP, while expression of GluA2 CTP modestly increased the total dendritic length and total branch tip number without changing branch density. Importantly, the bi-modal experience-dependent plasticity that has been observed in tectal inhibitory neurons (He et al., 2016) were disrupted by the disruption of excitatory inputs. These studies suggested differential effects of decreasing AMPAR-mediated fast excitatory synaptic transmission on structural plasticity in inhibitory and excitatory neurons.

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Poster

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Topic: A.06. Synaptogenesis and Activity-Dependent Development

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Title: The NuRD chromatin remodeling complex drives neural circuit development *In vivo*

Authors: ***K. K. HILL**[1], Y. YANG[1], T. YAMADA[2,1], T. E. HOLY[1], A. BONNI[1];
[1]Washington Univ. Sch. of Med., Saint Louis, MO; [2]Univ. of Tsukuba, Tsukuba, Japan

Abstract: Chromatin remodeling enzymes are thought to play key roles in brain development, but their relevance to in vivo neural circuit function remains poorly understood. We have

identified a role for the nucleosome remodeling and deacetylase (NuRD) chromatin remodeling complex in dendrite morphogenesis in granule neurons of the mouse cerebellar cortex. Conditional knockout of the core NuRD subunit, Chd4, impairs granule neuron dendrite pruning in vivo. To uncover the function of the NuRD complex in neural circuit function, we developed a technique for in vivo imaging of awake behaving mouse pups. Two-photon calcium imaging of head-fixed juvenile mice walking on a motorized treadmill reveals a sub-population of cerebellar granule neurons that is stimulus-responsive. Remarkably, conditional knockout of Chd4 results in hyperresponsivity of granule neurons to the sensorimotor treadmill stimulus. Our findings suggest the NuRD chromatin remodeling complex drives dendrite morphogenesis and sparse sensorimotor encoding in the cerebellum.

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Poster

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Nancy Lurie Marks Family Foundation

Title: Developmental switch in striatal adaptation to cortical hyperactivity

Authors: *R. PEIXOTO, W. WANG, J. LEVASSEUR, B. SABATINI;
Harvard Med. Sch., Boston, MA

Abstract: Despite the implication of basal ganglia (BG) dysfunction in multiple neurodevelopmental disorders the rules underlying the maturation of BG circuits remain poorly understood. Our previous work has shown that afferent activity is a major driver of glutamatergic synaptogenesis in striatal spiny projection neurons (SPNs) suggesting that perturbations of cortical activity during early postnatal stages can alter the normal development of corticostriatal circuits. Indeed, mice lacking Shank3 isoforms ($Shank3B^{-/-}$) exhibit increased corticostriatal network activity during the second postnatal week resulting in accelerated maturation of excitatory synapse development in SPNs. However, this early phenotype is opposite to the one observed in adult animals that exhibit depressed corticostriatal connectivity instead. To explore the causes underlying these phenotypic differences we tested how SPNs respond to increased

cortical activity during different stages of development and manipulated cortical activity by silencing cortical interneuron output via Cre-dependent deletion of the vesicular GABA transporter (vGAT). Interestingly, we found that chronic elevation of cortical activity from early postnatal stages recapitulates the phenotype found in Shank3B$^{-/-}$ animals, with increased connectivity early on and a subsequent reduction in adult stages. Moreover, elevation of cortical activity during adulthood for one week (P53-P60) was sufficient to depress corticostriatal connectivity whereas activation of the Gi-coupled DREADD hM4Di in layer 5 cortical neurons of adult Shank3B$^{-/-}$ mice partially rescued the corticostriatal depression found in adulthood. Together these results reveal distinct and dynamic adaptations of SPNs to cortical hyperactivity during different developmental stages and suggest that the abnormal developmental trajectory of corticostriatal connectivity caused by loss of Shank3 is caused by persistent cortical hyperactivity.

Disclosures: **R. Peixoto:** None. **W. Wang:** None. **J. Levasseur:** None. **B. Sabatini:** None.

Poster

677. Neural Circuit Activity and Maturation II

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 677.21/D11

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: CIHR

Heart and Stroke Foubdation Quebec

CHU Ste. Justine Foundation

Title: Exploring the role of p75NTR signaling pathway on GABAergic circuit maturation following neonatal hypoxia induced seizure

Authors: B. CHATTOPADHYAYA, M. BERRYER, D. DUFOUR-BERGERON, N. SANON, C. BOSOI, L. CARMANT, *G. DI CRISTO;
Res. Ctr., CHU Ste.justine-University of Montreal, Montreal, QC, Canada

Abstract: Perinatal hypoxic-ischemic encephalopathy is the most important cause of acute mortality and morbidity in newborns. The most common acute effect of hypoxic-ischemic encephalopathy is neonatal seizures, which are very often refractory to conventional seizure medications. Hypoxia-induced seizures (HIS) are associated with a high incidence of epilepsy as well as cognitive disabilities later in life. Despite the significant long-term morbidity of HIS in the neonates, there is currently no specific treatment. Further, our understanding of how HIS

changes the developmental trajectory of neuronal circuit development, and ultimately results in cognitive dysfunction, is still mostly unknown. Understanding the precise mechanisms by which HIS affects brain development, and how its effects can be ameliorated, can help us in designing the appropriate therapeutic approaches towards preventing the consequences of neonatal hypoxia.
Using a combination of molecular tools, electrophysiological recordings and imaging techniques, we studied the affects of HIS particularly on inhibitory GABAergic synapse development in rodent neocortex. We determined that HIS affects the maturation of distinct GABAergic interneuron populations, parvalbumin (PV)-positive and somatostatin (SOM)-positive interneurons differentially in the neocortex and in the hippocampus. In particular, we show that PV expressing interneurons in the neocortex remain immature. This correlates with a reduction in gamma oscillation power during exploration, and with impaired working memory and social novelty recognition. We are currently investigating the molecular mechanisms involved, specifically looking at the role of neurotrophin receptor (p75NTR) mediated signaling pathways in ameliorating the deficits induced by HIS on GABAergic circuit development.
All together, the knowledge we will gain from these experiments will pave the way for developing novel, targeted pharmacological interventions to prevent the severe and lasting consequences of perinatal HIS.
Funded by CIHR, Heart and Stroke Foundation (Quebec)

Disclosures: B. Chattopadhyaya: None. **M. Berryer:** None. **D. Dufour-Bergeron:** None. **N. Sanon:** None. **C. Bosoi:** None. **L. Carmant:** None. **G. Di Cristo:** None.

Poster

677. Neural Circuit Activity and Maturation II

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 677.22/D12

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: NIH Grant MH103861

Title: T-cadherin/Cdh13 expressed by hippocampal interneuron populations affects synaptic activity and memory

Authors: *J. LIU[1], H. BADIE-MAHDAVI[2], A. ROBERTS[3], J. SANES[4], B. RANSCHT[2]; [1]Sanford Burnham Prebys Med. Discovery Inst., LA Jolla, CA; [2]Sanford Burnham Prebys Med. Discovery Inst., La Jolla, CA; [3]The Scripps Res. Inst., La Jolla, CA; [4]Harvard Univ., Cambridge, MA

Abstract: A growing body of evidence implicates cell surface glycoproteins in regulating synapse formation, stability and function in the central nervous system (CNS). Glycosylphosphatidylinositol-linked T-cadherin (T for truncated; also Cadherin-13; *Cdh13*) is prominent in diverse structures of the CNS. Variations in the human *CDH13* gene have been associated with neuropsychiatric disorders, including addiction, bipolar-, autism spectrum- and attention deficit hyperactivity disorders. Mice generated to systemically lack Cdh13 gene expression show no overt neurological phenotypes under baseline conditions but display alterations in cue-based memory. In the hippocampus, T-cadherin immunoreactivity delineated pyramidal cell dendritic fields in stratum radiatum and lacunosum moleculare and the dendritic fields of dentate granule neurons. This pattern was reiterated in $Cdh13^{Cre};Rosa26^{LSL\text{-}tdTomato}$ reporter mice expressing the red fluorescent protein by recombination with Cre expressed in place of the first *Cdh13* coding exon. The tamoxifen-inducible reporter strain was used to determine the neuron populations expressing T-cadherin during hippocampal development. At all stages examined, reporter fluorescence was induced in $GAD67^{+}$ and $VGAT^{+}$ interneurons while neurons classified as excitatory did not show the red fluorescent protein marker. Sub-populations of interneurons expressing T-cadherin were further classified. In adult mice, $Cdh13^{Cre};Rosa26^{LSL\text{-}tdTomato}$ fluorescence was observed in interneurons expressing somatostatin $(SST)^{+}$ and vasoactive intestinal polypeptide (VIP), but not in major populations of other interneuron types. We could not discern $Cdh13^{Cre};Rosa26^{LSL\text{-}tdTomato}$ expression in association with CA1 pyramidal neurons at any postnatal stage examined. In acutely prepared brain slices from mice systemically lacking T-cadherin, CA1 pyramidal cell dendritic spines were reduced in number, and fewer spines reached maturity. This correlated with significant reductions in synaptic transmission and CA1 long-term potentiation. Our data suggest that T-cadherin regulates maturation/function of interneuron populations intrinsic to the hippocampus and thereby modulates pyramidal neuron synaptic activity associated with aspects of hippocampal-based memory formation, processing or retrieval.

Disclosures: **J. Liu:** None. **H. Badie-Mahdavi:** None. **A. Roberts:** None. **J. Sanes:** None. **B. Ranscht:** None.

Poster

678. Sensory Systems Development: Activity and Circuits

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 678.01/D13

Topic: A.08. Development of Motor, Sensory, and Limbic Systems

Support: JSPS Kakenhi 2411068

JSPS Kakenhi 25221001

Grant-in-Aid 25117004

Strategic International research Cooperative Program

Title: Gap junctions in early postnatal excitatory neurons regulate spine density and maturation of response reliability

Authors: *K. HAYASHI, K. OHKI;
The Univ. of Tokyo, Tokyo, Japan

Abstract: Gap junctions in neocortex excitatory neurons have been demonstrated to interconnect the cells only during the development stage and enable propagation of electrical activity and small molecules and are suggested to have essential roles in the development of neuronal circuits. However, the roles of gap junctions in the development of neuronal circuits have remained to be elucidated, mainly due to technical difficulties in regulating the expression of gap junctions without affecting migration of neurons to the cortical plate.
Here we achieved Tet-inducible knockdown (KD) of connexin-43 only during the early postnatal stage to avoid the effect of connexin-43 KD on the migration of neurons. The KD vector was transfected to V1 by *in utero* electroporation at E15.5 to induce the shRNA expression in layer 2/3 excitatory cells, and the mice were administered with Dox to repress the expression of the shRNA with an exception of a developing period from P2 to P14. We found that connexin-43 KD significantly decreased spine density of KD neurons. Further, we examined visual functions of connexin-43 KD neurons by using *in vivo* two photon calcium imaging. We found that connexin-43 KD resulted in sharper orientation selectivity. Furthermore, we found that reliability of the visual response was decreased by the KD. The higher visual selectivity and the lower response reliability in connexin-43 KD neurons may be attributable to a decrease in spine density. These results suggest that connexin-43 before visual experiences is essential for neuronal circuitry development and regulates the spine density for reliable computation.

Disclosures: K. Hayashi: None. **K. Ohki:** None.

Poster

678. Sensory Systems Development: Activity and Circuits

Location: Halls B-H

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Topic: A.08. Development of Motor, Sensory, and Limbic Systems

Support: NIH P30-GM-32128

Title: A role of presenilin in the developing visual circuit of Xenopus tadpole

Authors: *Z. LIU[1], A. M. THAKAR[2], K. G. PRATT[2];
[2]Zoology and Physiology, Program in Neurosci., [1]Univ. of Wyoming, Laramie, WY

Abstract: Presenilin (PS) is an interesting molecule that was first identified, and named, in the context of Alzheimer's disease, but is now known to carry out a myriad of functions that are important during development. As the catalytic component of γ-secretase, PS is responsible for cleaving a wide range of substrates that are crucial for many phases of nervous system development, including neurogenesis, differentiation, and axon guidance. This suggests a global and multifaceted role for this molecule in the development of neural circuits. Here, to provide a comprehensive understanding of the roles PS plays in circuit development we use the Xenopus tadpole retinotectal projection as our model. This projection is the major component of the amphibian visual system. This model system allows for the function of a protein to be characterized across all stages of neural circuit development at the cell, circuit, and behavioral levels. First, western blot studies confirmed that PS is expressed in the tadpole optic tectum during the time when the retinotectal circuit is forming. To test the role of PS during the development of this circuit, PS function was inhibited globally by adding the PS blocker L685,458 (5μM) to the tadpoles' rearing solution during development, or by electroporating a PS morpholino into the tectum to knock down PS expression specifically in the postsynaptic tectal neurons. We found that blocking PS function using either of these approaches significantly compromised visual avoidance behavior, which was quantified using an established moving dot test (control: 65.6% ± 5.9%, n=25; PS morpholino: 33.3% ± 4.9%, n=30). This suggests deficits in visual system function. To further identify the underlying pathology at the circuit level, we performed in-vivo whole cell electrophysiological recordings from PS-inhibited neurons. We found that PS morpholino-transfected neurons displayed significantly decreased peak current amplitudes in response to light-activated RGC input (control: 72.07 ± 11.01 pA, n=15; PS morpholino: 28.87±3.43 pA, n=16) while passive electrical properties such as resting membrane potential, input resistance, and capacitance were unchanged compared to control. This indicates that the decreased light responses could be due to compromised synaptic transmission. Preliminary data shows a modest yet significant increase in paired-pulse facilitation at the retinotectal synapse, indicating decreased probability of presynaptic transmitter release. Thus far, our results suggest a role for PS in the normal development and function of the tadpole visual system.

Disclosures: Z. Liu: None. **A.M. Thakar:** None. **K.G. Pratt:** None.

Poster

678. Sensory Systems Development: Activity and Circuits

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Title: Cortical layer 6b neurons selectively innervate higher order nuclei in the thalamus

Authors: A. HOERDER-SUABEDISSEN[1], *A. L. UPTON[1], E. L. GRANT[1], K. V. KORRELL[1], S. VISWANATHAN[2,3], P. O. KANOLD[2], Y. KIM[4], Z. MOLNAR[1];
[1]Univ. of Oxford, Oxford, United Kingdom; [2]Biol., Univ. of Maryland, College Park, MD; [3]Janelia Res. Campus, Ashburn, VA; [4]Col. of Med., Penn State Univ., Hershey, PA

Abstract: We studied the targeting of layer 6b projections to the thalamus by using a transgenic mouse line (Drd1a-Cre). The cortical Cre expression is selective to layer 6b and starts from early postnatal ages and continues to adulthood. The labelled cortical neurons in the Drd1a-Cre::tdTomato line show an antero-posterior and medio-lateral gradient of expression level. 15%±7 (mean±s.d.) of NeuN expressing neurons in layer 6b is labelled by Drd1a-Cre::tdTomato in S1 at P8. The labelled neurons are immunoreactive for Ctgf, neuroserpin and Cplx3. They do not co-localise with Lpar1-eGFP labelled subplate cells in triple transgenic mice. Using the Drd1a-Cre::tdTomato mouse we studied the targeting of the layer 6b projections from the entire cortical mantle to the thalamus and established that these projections selectively innervate the higher order thalamic nuclei (including PO, LP). This is similar to layer 5 thalamic projections, and in contrast to layer 6a. No labelled projections were found contributing to the corpus callosum. Using Cre-dependent eYFP expressing AAV virus tracing from S1 revealed that layer 6b neurons from this region project to PO, whereas those from M1 do not. We used serial two-photon tomography based whole brain imaging in cellular resolution and quantified axonal projection pattern in the entire brain. The morphology and size of the terminals was compared

with 6a and layer 5 thalamic terminals. The layer 6b subcortical projections do not form branches in thalamic reticular nucleus, similar to layer 5 projections. Our finding that layer6b projections selectively target higher order thalamic nuclei and develop a similar thalamic innervation pattern to layer 5 is an unexpected and novel discovery that has to be integrated into the current schemes of thalamocortical communication systems. Acknowledgements: ZM's laboratory is supported by MRC (G00900901), BBSRC (BB/1021833) and The Wellcome Trust (092071/Z/10/Z). EG held an MRC Doctoral Studentship. YK is supported from a NARSAD Young Investigator Grant and NIH U01MH105971; PK from NIH R01DC009607

Disclosures: **A. Hoerder-Suabedissen:** None. **A.L. Upton:** None. **E.L. Grant:** None. **K.V. Korrell:** None. **S. Viswanathan:** None. **P.O. Kanold:** None. **Y. Kim:** None. **Z. Molnar:** None.

Poster

678. Sensory Systems Development: Activity and Circuits

Location: Halls B-H

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Program#/Poster#: 678.04/D16

Topic: A.08. Development of Motor, Sensory, and Limbic Systems

Support: 国家重点基础研究发展计划（973计划），课题编号：2011CB504402

Title: Roles of Celsr3 in central projections of dorsal root ganglion neurons

Authors: ***W. FEIFEI**[1], L. ZHOU[2];
[1]Guangdong-hongkong-Macau Inst. of CNS Regene, Guangdong, China; [2]Guangdong-Hongkong-Macau Inst. of CNS Regeneration,Jinan Univ., Guangzhou, China

Abstract: Dorsal root ganglion (DRG) neurons relay nociceptive and thermoceptive, mechanoreceptive, and proprioceptive information to the central nervous system via different afferent axons. Different central projections establish interactions with different targets in the spinal cord, such as large proprioceptice fibers transiently synapse with the spinal motoneuorns at early development. Atypical cadherein *Celsr3* plays a critical role in brain wiring including forebrain, hippocampus and spinal cord. *Celsr3* mRNA is highly expressed in the DRGs, but its function in the central projections of DRGs is still not known. Here we study this issue by inactivating *Celsr3* in DRGs with *Wnt1-Cre*. Using *Celsr3-GFP* transgenic mice, *Celsr3* is identified to be expressed in different DRG cells. Upon Wnt1-Cre activation, *Celsr3* is conditionally removed in DRG cells and some sensory neurons in the dosal spinal cord. In *Wnt1-Cre;Celsr3*$^{f/-}$ mutants, the sensory process is abnormal; the distribution of central projections of DRG neurons is studied using DiI tracing and immunostaining and showed different from the control samples. Furthermore, Transient synapses between Ia afferent fibers and spinal

motoneurons behave somewhat abnormalities in the mutant. In conclusion, *Celsr3* is involved in axonal targeting of the DRGs in mice.

Disclosures: W. Feifei: None. **L. Zhou:** None.

Poster

678. Sensory Systems Development: Activity and Circuits

Location: Halls B-H

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Topic: A.08. Development of Motor, Sensory, and Limbic Systems

Support: Marie Slodowska Curie Horizon 2020

ERC (Consolidator)

Title: Early GABAergic microcircuits and cortical dynamics

Authors: *L. MODOL VIDAL[1], V. SOUSA[2], A. MALVACHE[2], T. TRESSARD[2], A. BAUDE[2], R. COSSART[2];
[1]INMED, INSERM U901, marseille, France; [2]INMED, Inserm, Marseille, France

Abstract: The onset of electrical activity in a developing neural network is characterized by the acquisition of spontaneous oscillatory coordinated activity between large numbers of maturing neurons. Such coordinated neuronal activities play a pivotal role in the assembly of neuronal circuits and are characteristic of most developing neural systems as they have been observed in a wide array of peripheral and central tissues. Therefore, to understand how neural circuits form in the normal and pathological brain it is essential to understand the molecular and cellular mechanisms of synchronization in maturing cortical networks. Functional maturation of GABAergic neurons has been described to shape cortical dynamics, plasticity and synaptic wiring during the course of pre- and -postnatal development. For instance, the emergence of Giant Depolarizing Potentials (GDPs) has been shown to be driven by GABAergic transmission . Although GDP appearance has been described to depend on long-range GABA ‘‘hub’’ neurons (high functional connected neurons) in the hippocampus and to be responsible to orchestrate synchrony of hippocampal development nothing is known concerning their role in the maturation of the neocortex. This is an important issue given that both structures share common developmental programs including a similar sequence for the emergence of population coherence, but also since recent evidence indicates important differences especially regarding the origin of GABAergic neuron diversity. Therefore, understanding the function of these GABA hub neurons and their origin is a hallmark to elucidate mechanisms underlying cortical

maturation. To this aim, we have applied a multidisciplinary strategy to our experiments including functional multineuron calcium imaging *in vitro* and *in vivo*, online computational analysis and electrophysiology, as well as mouse genetics in order to approach and to analyse the changes in cortical activity during the maturation of mammalian cortical networks.

Disclosures: L. Modol Vidal: None. **V. sousa:** None. **A. Malvache:** None. **T. Tressard:** None. **A. Baude:** None. **R. Cossart:** None.

Poster

678. Sensory Systems Development: Activity and Circuits

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Topic: A.08. Development of Motor, Sensory, and Limbic Systems

Support: NIH-NEI Grant 2T32EY015387-11

NSF Grant GRFP DGE -1148897

NIH grant 5R01EY022987-03

Title: Genetic and epigenetic regulation of the cortical phenotype: The effects of early bilateral enucleation on epigenetic and genetic modifications in developing neocortex

Authors: K. FOREMAN[1], D. RAMAMURTHY[2], C. WELLER[2], L. A. KRUBITZER[2], *D. S. STOLZENBERG[1];
[1]Psychology, [2]Ctr. for Neurosci., Univ. of California Davis, Davis, CA

Abstract: Early experience plays a critical role in the development of the nervous system, particularly the neocortex. For example, early exposure to persistent sensory stimuli, or loss of sensory inputs critically impacts cortical map formation and connectivity of cortical fields. While the types of modifications to the neocortex that occur when sensory input is altered during development have been well described, the underlying mechanisms that give rise to these changes are not well understood. In the current investigation we utilized a unique animal model, the small South American short-tailed opossum (*Monodelphis domestica*), to examine how early and complete loss of vision alters the expression pattern of genes involved in the formation of cortical fields and cortical connections. Importantly, the opossum is born extremely immature, so that bilateral enucleations can be made well before the retinal ganglion cells have reached the diencephalon and before thalamocortical axons have reached their targets in the neocortex. Thus, using this model organism we can examine how this very early loss of vision alters the expression of key genes involved in axonal guidance, cortical arealization, and cortical

connectivity within primary somatosensory and visual cortices at several key developmental stages. In these studies, *Monodelphis domestica* were bilaterally enucleated at P4 (equivalent to embryonic day 15 in mice) and the effects of this manipulation were examined just after eye opening (P36). The expression of several genes (*Ephrin A5, COUP-TF1, RZRβ, ID2, Cad8,* and *EphA7*) was measured by real-time qPCR from microdissected primary somatosensory and visual cortical tissue. All microdissections were histologically verified. As a first step toward understanding the mechanisms through which early sensory experience helps construct the developing cortex, we also examine epigenetic modifications that may function to program the expression of these genes involved in directing cortical development and connectivity.

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Poster

678. Sensory Systems Development: Activity and Circuits

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Program#/Poster#: 678.07/D19

Topic: A.08. Development of Motor, Sensory, and Limbic Systems

Title: Comparison of postnatal cortical development between primary auditory and somatosensory cortexes in the mouse

Authors: *M. CHANG, S. ISHIZAWA, H. D. KAWAI;
Dept. of Bioinformatics, Soka Univ., Hachioji, Tokyo, Japan

Abstract: Sensory cortices are considered to possess similar laminar architecture, however earlier studies by Andersen et al. (*Brain Res*., 1252, 130-142, 2009) had suggested that primary auditory cortex (A1) might differ from primary somatosensory and visual cortexes (S1 and V1) based on the staining pattern observed using cytochrome oxidase and acetylcholinesterase. In the past, we lacked information that could help us define each layer and neuronal subtypes using specific genetic markers. However, today, such information is available for some of the genes (Molyneaux et al., *Nat. Rev. Neurosci.*, 8, 427-437, 2007).
In this experiment, we used different specific lamina markers to study the lamination between A1 and S1 during postnatal development. We used antibodies against Cut-like homeobox 1 (Cux1), COUP-TF-interacting protein 2 (Ctip2), and Forkhead-box protein 2 (Foxp2) to identify layers 2-4, 5, and layer 6, respectively. The results show that the lamination between the two cortices were significantly different. At postnatal day (PD) 5, the cortical proportion of layer 5 and that of layer 6 were ~15% wider and ~10% narrower in presumed A1 compared to presumed S1, respectively. This contrasting proportional difference remained throughout development until

the adulthood. Thus, from PD 10 through PD 60, layer 5 proportion was ~13% wider in A1 compared to S1. The difference in layer 6 proportion became slightly smaller with ~7% narrower laminar thickness in A1 than S1. The proportion of layers 2-4 was ~10% narrower in A1 compared to S1.
Ctip-2 immunopositive cells, which are reportedly subcortical projecting neurons, were distributed sparsely in A1 at any ages, while they distributed more homogeneously in the lower portion of layer 5 in S1. Retrograde labeling of neurons projecting to contralateral A1 (cA1) and ipsilateral inferior colliculus (iIC) indicated that the labeled neurons were intermingled in layer 5 of A1. Some of the commisural A1 projection neurons were also present in FoxP2-defined layer 6. iIC projection neurons were located in layer 5 with Ctip2-positive neurons below the upper layers defined by Cux1 and above layer 6 defined by Foxp2.
Our studies suggest that laminar thickness and projection neuron distributions in A1 differ from those in S1.

Disclosures: **M. Chang:** None. **S. Ishizawa:** None. **H.D. Kawai:** None.

Poster

678. Sensory Systems Development: Activity and Circuits

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Topic: A.08. Development of Motor, Sensory, and Limbic Systems

Support: Agence National Recherche: ANR-15-CE16-0016-01

Title: Anatomical, molecular and functional characterization of rhombomere 4-derived sensorimotor sub-circuits in the mouse brainstem

Authors: ***M. DI BONITO**, E. SETTI, M. STUDER;
iBV Inst. de Biologie Valrose, CNRS UMR7277, Inserm U1091, UNS Univ. Nice Sophia Antipolis, Nice, France

Abstract: Neuronal networks relay sensory information and process motor responses to control vital functions. The sensorimotor circuits are established during development and depend on spatially- and temporally-ordered sequence of neuronal specification, migration and connectivity. Cell lineage studies have started to correlate early rhombomeric subdivisions to adult neuronal connectivity maps in the brainstem demonstrating that regional patterning along the anteroposterior (AP) axis and neuronal subtype specification along the dorsoventral (DV) axis intersect to specify neuronal fates. *Hox* genes, besides conferring AP identity during hindbrain segmentation, regulate the specification, the stereotypic neuronal migration and axon

pathfinding as well as topographic connectivity of different sensorimotor neurons along the DV axis in a rhombomere-specific manner. We have used a *b1r4-Cre* mouse line to genetically label rhombomere 4 (r4) and followed all r4-derived neuronal populations and axonal tracts at different stages from their embryonic origin to the adult final location and targets. We have demonstrated that a rhombomere-specific *Hox* code controls the assembly of distinct functionally segregated sub-circuits in the developing auditory brainstem. R4 contributes to the majority of the auditory nuclei required for sound transmission and amplification, and to the establishment of two efferent feedback sub-circuits involved in the protection from acoustic overstimulation. In the vestibular system, r4 gives rise mainly to the vestibulospinal projection neurons and in particular to the lateral vestibulospinal tract, the principal pathway that conveys vestibular information to limb-related spinal motor circuits. Moreover, r4 contributes to the specific topographic trigeminal pathway to the thalamus associated with the r4-derived spinal trigeminal oral sub-nucleus. In this study, we use a novel *r4-Flippase* mouse and several subtype-specific *Cre-recombinase* lines of different DV domains to characterize by intersectional fate mapping the specific contribution of r4 sub-domains to distinct sensorimotor systems. The intersectional genetic strategy will enable to label and capture selected neuronal populations and to identify, through genomic approaches, novel factors of single rhombomere-specific DV domains. Our overall aim is to dissect the molecular and cellular mechanisms allowing proper cell fate specification, migration and connectivity of sensorimotor neurons originating from rhombomere 4.

Disclosures: **M. Di Bonito:** None. **E. Setti:** None. **M. Studer:** None.

Poster

678. Sensory Systems Development: Activity and Circuits

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Topic: A.08. Development of Motor, Sensory, and Limbic Systems

Support: NSF Grant 1451480

NIH Grant MH078829

Title: Dynamic changes in brain network activation during emotional face processing in the developing brain

Authors: ***C. STAMOULIS**[1], P. DINARDO[3], A. WESTERLUND[3], C. A. NELSON, III[2]; [2]Developmental Med., [1]Harvard Med. Sch., Boston, MA; [3]Developmental Med., Boston Children's Hosp., Boston, MA

Abstract: There is substantial evidence that adult brains are organized into parsimonious and optimally connected small-world and scale-free networks, which facilitate efficient processing of sensory information and cognitive performance. In contrast, little is known about the dynamic development of these networks in early life, their progressive optimization and neural computation across incompletely myelinated and redundantly connected circuits. Furthermore, there is limited systematic evidence that core networks, which are involved in cognitive function that is critical for survival, may develop at distinct rates from those involved in higher-level function. Using longitudinally acquired electrophysiological (EEG) data measured at <12 months and 36 months from 58 typically developing infants, this study investigated changes in the organization of distributed functional network activation in response to emotionally salient faces (happy, angry, fearful and neutral (only at 36 months)) during early development. A significant increase in network connectivity in response to fearful faces (compared to happy and angry faces) was estimated both at <12 months ($p<0.001$) and 36 months ($p<0.01$) across neural oscillations. This indicates that the mechanisms underlying network activation in response to fear may be in place at birth or the first few months of life. However, differential clustering in connectivity was also estimated in oscillation-specific networks, including higher connectivity in frontal areas in the gamma network and higher connectivity in parieto-occipital areas in the alpha network. A significant decrease in the number of network connections was estimated from <12 to 36 months across oscillations ($p<0.001$). Finally the lowest number of network connections was estimated in response to neutral faces ($p<0.0001$). These findings suggest that differential network activation in response to fearful faces occurs as early as 5 months and persists consistently during the first 3 years of life, although functional network topologies change dynamically during this period becoming increasingly sparse and clustered in a frequency-specific manner.

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Poster

678. Sensory Systems Development: Activity and Circuits

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Program#/Poster#: 678.10/D22

Topic: A.08. Development of Motor, Sensory, and Limbic Systems

Support: NIH 1R01-EY025627-01

Title: Theoretical models of visual map alignment in the superior colliculus.

Authors: *R. A. TIKIDJI-HAMBURYAN[1], T. EL-GHAZAWI[2], J. W. TRIPLETT[3];
[1]Inst. of Massively Parallel Application and Computer Technology, Sch. of E, [2]Sch. of Engin. and Applied Sci., George Washington Univ., Washington, DC; [3]Ctr. for Neurosci. Res., Children's Natl. Hlth. Syst., Washington, DC

Abstract: In the visual system, neuronal connections are organized topographically to preserve the spatial order of the visual scene. In associative visual centers, topographic projections from multiple areas must be aligned to facilitate integration. However, the mechanisms by which alignment is achieved remain poorly understood. The superior colliculus (SC) integrates visual inputs from retinal ganglion cells (RGCs) and Layer 5 neurons of the primary visual cortex (L5-V1), each of which are organized topographically and in register with one another. Previous studies suggest that RGCs instruct L5-V1 alignment in a manner dependent on spontaneous retinal activity, however, the underlying mechanism of activity-dependent instruction remains unclear. To explore possible mechanisms and generate testable hypothesis, we have developed novel computational framework to describe visual map alignment in the SC. Similar to existing models of retinocollicular map formation, our model of alignment consists of three major components: chemoaffinity (interaction between Ephin-A/B and Eph-A/B), axon competition and activity dependent plasticity. We were able to adopt existing chemoaffinity and axon competition components with subtle changes to replicate *in vivo* organization of L5-V1 inputs. To explore the mechanism of activity-dependent alignment, we developed two models, each of which are able to replicate *in vivo* experimental findings. The first model is based on assumption that spiking activity of SC neurons is driven exclusively by RGC inputs, with no contribution from V1 axon activity. This model effectively simulates map alignment under wild type (WT) conditions, as well as under conditions in which the retinal map is duplicated, as in *Islet2-EphA3* knock-in mice ($Isl2^{EphA3}$) and disruption of retinal activity, as in mice lacking the beta2 subunit of the nicotinic acetylcholine receptor ($\beta2^{-/-}$). However, this model fails to replicate *in vivo* findings in combination $Isl2^{EphA3}/\beta2^{-/-}$ mice unless the spatial correlation parameter is increased 7-fold over reported values in the retina. In a second model, we assumed that SC neurons are driven by both RGCs and V1 inputs, but RGCs inputs significantly stronger. Remarkably, this model is able to simulate L5-V1 projection patterns in the SC of WT, $Isl2^{EphA3}$, $\beta2^{-/-}$ and $Isl2^{EphA3}/\beta2^{-/-}$ mice with minimum additional assumptions. We then used embarrassingly parallel computing on 1048 cores cluster to study robustness of both models. These novel computational models of visual map alignment in the SC suggest an activity-dependent mechanism of map alignment in which V1 inputs contribute to SC spiking activity during development.

Disclosures: R.A. Tikidji-Hamburyan: None. **T. El-Ghazawi:** None. **J.W. Triplett:** None.

Poster

678. Sensory Systems Development: Activity and Circuits

Location: Halls B-H

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Program#/Poster#: 678.11/D23

Topic: A.08. Development of Motor, Sensory, and Limbic Systems

Support: 2014CB846100

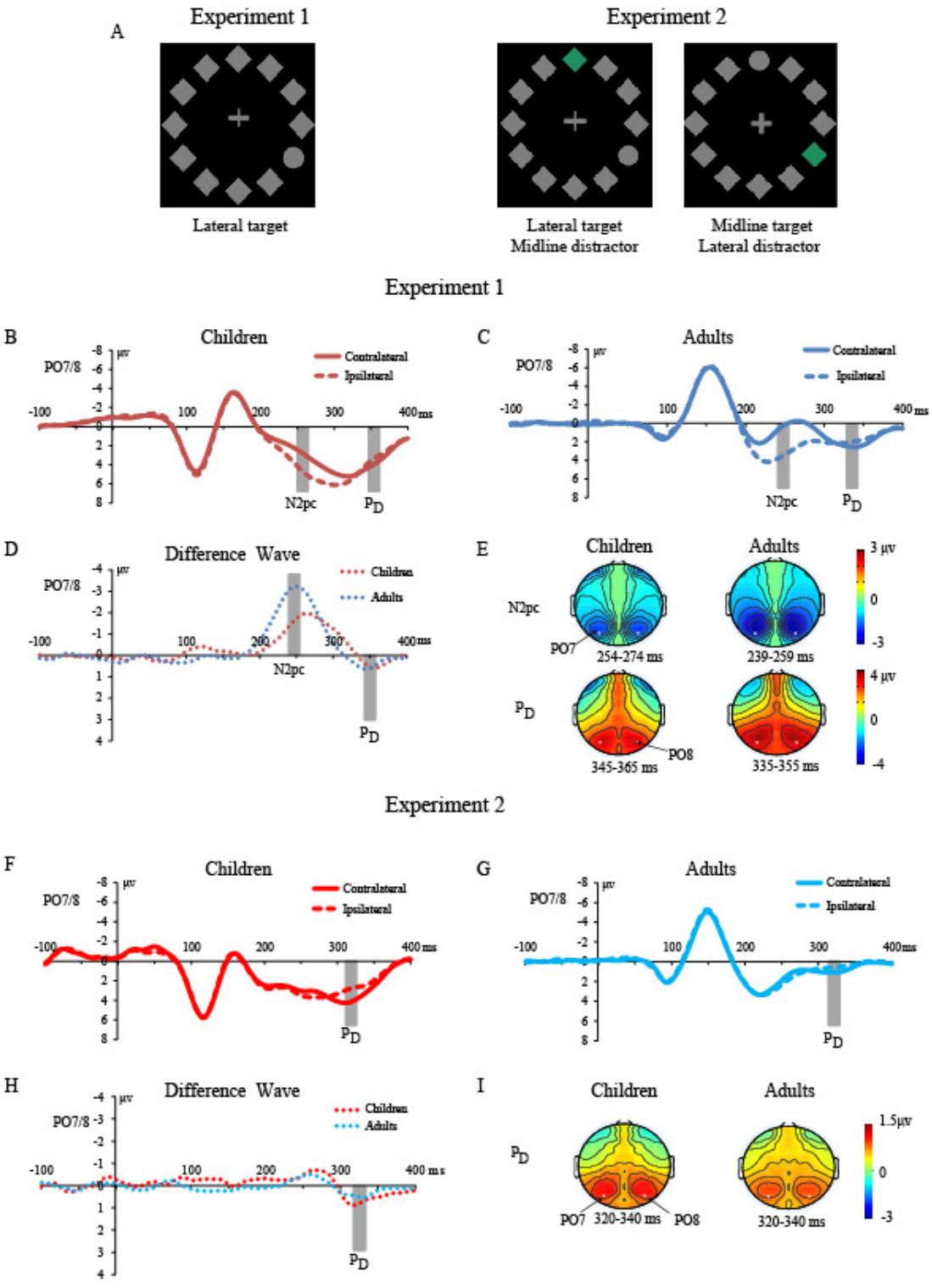
31271203

81371496

Title: Distinct developmental patterns in attentional selection and suppression

Authors: *E. WANG[1], M. SUN[1], Y. TAO[1], J. HUANG[1], L. SUN[2], Y. SONG[1];
[1]State Key Lab. of Cognitive Neurosci. and Learning, Beijing Normal Univ., Beijing, China;
[2]Peking Univ. Sixth Hosp. / Inst. of Mental Hlth., Beijing, China

Abstract: Searching through a visual scene requires a number of complex processes, such as covert spatial attention, which must be utilized to enhance processing of the attended items while simultaneously irrelevant items must be ignored. Although covert visual spatial attention is very important in many everyday situations, little is known about whether and how covert spatial attention differs in children compared to adults. The current study sought to identify the neurophysiological bases of development in covert spatial attention, focusing on electroencephalographic (EEG) markers of attentional selection (N2pc) and suppression (P_D). EEG data were collected from healthy young adults (aged 20-28 years) and typically developing children (ages 9-15 years), while they searched for a shape singleton target either with the absence (Experiment 1) or presence (Experiment 2) of a task-irrelevant color singleton distractor. The results showed that the shape target elicited a prolonged and smaller N2pc in children (n = 25) compared to adults (n = 28) in Experiment 1, and the N2pc delay might be caused by the early P1 component. Moreover, the target-elicited N2pc was followed by a similar positivity in both children and adults. In Experiment 2, we replicate the reduced target-elicited N2pc in children. Counterintuitively, the salient-but-irrelevant color distractor elicit a larger P_D in children (n = 22) than in adults (n = 31). We found no evidence for a correlation between the reduced target-elicited N2pc and the increased distractor-elicit P_D in children. Our results provide neurophysiological evidence that covert spatial attention in children of 9-15 years old is still undergoing significant development. Compared to adults, children deploy insufficient attention resources to the targets and use more attentional suppression to resist to the silent-but-irrelevant distractors. However, the development of target selection and distractor suppression might depend on distinct cognitive mechanisms.



Disclosures: **E. Wang:** None. **M. Sun:** None. **Y. Tao:** None. **J. Huang:** None. **L. Sun:** None. **Y. Song:** None.

Poster

678. Sensory Systems Development: Activity and Circuits

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Topic: A.08. Development of Motor, Sensory, and Limbic Systems

Support: NIH GrantEY011261

Title: Calibration of visual map through bimodal NMDAR signal

Authors: *M. HIRAMOTO[1], H. CLINE[2];
[1]Mol. & Cell. Neurosci., [2]The Scripps Res. Inst., San Diego, CA

Abstract: The optical axis in the eyes varies between individuals, however, the anteroposterior axis of the visual field map in the brain precisely matches the spatial axis of the outer world. It suggests that sensory experience calibrates the antero-posterial axis of the visual field map. The anteroposterior perception is a feature of the bilaterally-symmetric animals. It predicts that the anteroposterior axis in the visual field map is calibrated utilizing some features of the bilaterally-symmetric animals. We previously found that the forward-directed optic flow, unique to the bilaterally-symmetric, calibrates the anteroposterior organization in the visual field map. In the mechanism, the retinal ganglion axons (RGCs) activated earlier are connected to the cells in the brain that perceive anterior vision. Thus, the anterior-posterior axis in the space is transformed into the sequential order of RGC activities, which is further transformed into the sequential projection of RGC axons, in a Spatial-Temporal-Spatial (STS) transformation manner. This temporo-spatial linkage reflects the natural situation in which objects in the optic flow move in the anterior to posterior direction Hiramoto & Cline, 2014). NMDARs are a key molecule that converts the sequential order of synaptic transmission into the directionality of synaptic plasticity through its bimodal properties, suggesting that NMDAR activity is a core event that links the sequential order of retinotectal synaptic transmission with the directionality of RGC axon movement. To test this, we stimulated two groups of RGC axons in a sequence and analyzed the retinotectal axon branch tip shifts in the tectum while manipulating NMDAR activity. We found that attenuating NMDAR inverts the directionality of branch shift induced by temporally ordered convergent inputs. This shows the role of NMDAR in the transformation of the sequential order of afferent activity in the temporal domain into the sequential spatial organization of the visual field map and the potential to change the transformation rule through the attenuation of NMDAR activity in other circuits.

Disclosures: **M. Hiramoto:** None. **H. Cline:** None.

Poster

678. Sensory Systems Development: Activity and Circuits

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NS086368

Title: Mechanistic insights into agonist binding domain mutations in NMDA receptors underlying neurodevelopmental disorders

Authors: *S. BHATTACHARYA[1], S. A. SWANGER[1], W. CHEN[2], K. L. STRONG[2], P. BURGER[3], G. WELLS[2], A. TANKOVIC[2], C. HU[2], H. KUSUMOTO[2], J. J. MILLICHAP[4], S. F. TRAYNELIS[2], H. YUAN[2];
[1]Pharmacol., [2]Emory Univ., Atlanta, GA; [3]Med. Univ. of South Carolina, Charlseton, SC; [4]Northwestern Univ. Feinberg Sch. of Medicine, and Ann & Robert H. Lurie Children's Hosp. of Chicago, Chicago, IL

Abstract: Ligand-gated ion channel *N*-methyl-*D*-aspartate receptors (NMDARs) are major excitatory neurotransmitter receptors in the mammalian brain. Mutations in the genes encoding NMDAR subtypes GluN2A/2B (*GRIN2A*/*2B*) are implicated in neurological diseases including epilepsy and intellectual disability. NMDARs have critical developmental and functional roles in the central nervous system. We have investigated the mechanisms by which NMDAR function, structure, and expression are altered by 25 missense mutations or rare (less than 1%) variants in the GluN2A and GluN2B agonist binding domains, which also include 3 unpublished mutations. Most of the mutations in *GRIN2A* were identified in patients with epilepsy or seizure disorders. When these missense mutations were introduced into recombinant human NMDAR subunits, they caused significant decreases in receptor expression and/or function. Furthermore, several

mutations in both *GRIN2A* and *GRIN2B* increased functional properties of NMDARs but reduced cell surface expression, which in the absence of homeostatic compensatory mechanisms might cause an overall reduction in NMDAR activity. It is important to note here that *GRIN2A* and *GRIN2B* mutations affected NMDARs through related mechanisms, but were associated with different neurological disorders and had different response to NMDAR modulators. Taken together, this study elucidates mechanisms by which mutations in both GluN2A and GluN2B alter the agonist binding domain and could be relevant for the different disorders observed in patients with *GRIN2A* vs *GRIN2B* mutations.

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Poster

678. Sensory Systems Development: Activity and Circuits

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Topic: A.08. Development of Motor, Sensory, and Limbic Systems

Support: NIH Director's New Innovator Award (DP2 EY024504-01)

Searle Scholars Award and a Klingenstein Fellowship

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Title: A redefined critical period for visual acuity

Authors: *M. F. DAVIS[1], D. X. FIGUEROA VELEZ, 92697[2], S. P. GANDHI[2];
[1]Neurobio. & Behavior, [2]Neurobio. and Behvaior, UC Irvine, Irvine, CA

Abstract: It is known that early visual deprivation leads to impaired vision in adulthood. It has also been shown extensively that monocular deprivation during a defined developmental critical period in visual cortex results in a phenomenon known as ocular dominance (OD) plasticity. Until now OD plasticity has been assumed to be synonymous with the critical period for the development of visual acuity. Here we show that although related, the critical period for visual acuity is distinct from the critical period for ocular dominance plasticity. The critical period for visual acuity occurs much earlier than previously thought. Brief monocular deprivation before the onset of OD plasticity (about 1 week after eye opening) produces an unexpected, long-lasting reduction in cortical acuity, whereas equivalent deprivation during OD plasticity has no persistent effect. Thus the well-studied critical period for OD plasticity is not coincident with the critical period for acuity development. We also show that enhancement of intracortical inhibition at this early time point, previously shown to activate precocious OD plasticity, disturbs the development of acuity. Further, early visual deprivation also altered subsequent OD plasticity, making it resemble adult plasticity. Thus although distinct, the acuity critical period and the OD plasticity critical period are clearly linked. Taken together, these results show that the critical period for visual acuity occurs earlier than previously thought, but is important for the subsequent development of normal OD critical period plasticity. Other developmental disorders of the brain may also involve early periods of vulnerability that precede the expression of measuable plasticity and overt disorganization of neural function.

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Poster

678. Sensory Systems Development: Activity and Circuits

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Program#/Poster#: 678.15/D27

Topic: A.08. Development of Motor, Sensory, and Limbic Systems

Title: CPEB3-regulated megf10 expression controls retinal mosaic spacing of starburst amacrine cells

Authors: *Y.-S. HUANG, Y.-P. CHEN, K.-S. BAI;
Academia Sinica/Institute of Biomed. Sci., Taipei, Taiwan

Abstract: Cytoplasmic polyadenylation element binding protein 3 (CPEB3) regulates target RNA translation in neurons. Here, we examined CPEB3 distribution and function in the retina.

CPEB3 is expressed in retinal neurons, including those located in the inner nuclear layer (INL) and ganglion cell layer (GCL), but not in photoreceptors and retinal pigment epithelia. Retinal neurons display spatial arrangements in order. Many individual subtypes are organized non-randomly in patterns called mosaics. Among them, CPEB3 was detected in cholinergic starburst amacrine cells (SACs) and calbindin-positive horizontal cells (HCs). Despite the presence of CPEB3 in both populations of SACs, OFF SACs in the INL and ON SACs in the GCL, CPEB3-knockout (KO) retina showed aberrant mosaic spacing only in ON SACs. Molecular characterizations identified that translation of multiple epidermal growth factor 10 (*Megf10*) RNA was suppressed by CPEB3 during the first week of postnatal development when MEGF10 is restrictedly expressed in SACs and mediates homotypic repulsive interactions for mosaic arrangement. Thus, elevated MEGF10 expression in the absence of repressor CPEB3 likely accounts for defective spatial organization of ON SACs.

Disclosures: **Y. Huang:** None. **Y. Chen:** None. **K. Bai:** None.

Poster

678. Sensory Systems Development: Activity and Circuits

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Topic: A.08. Development of Motor, Sensory, and Limbic Systems

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Professional Staff Congress-City University of New York (PSC-CUNY)

Faculty Research Grant II- American University of Sharjah

Title: Postnatal refinement of feedforward projections in ferret visual cortex.

Authors: *R. KHALIL[1], V. C. RAMIREZ[2], J. B. LEVITT[2];
[1]BCE department, American Univ. of Sharjah, Sharjah, United Arab Emirates; [2]Biol. Dept., The City Col. of New York, New York, NY

Abstract: Visual cortical areas in the adult mammalian brain are linked by a network of interareal feedforward (FF) and feedback (FB) circuits that refine from an immature state. We

studied the postnatal refinement of FF projections from ferret primary visual cortex (V1) to multiple cortical targets during the period from just before eye opening (4 weeks) to 10 weeks. Our aim was to determine (a) whether the developmental refinement of FF projections parallels that of FB cortical circuits, and (b) whether FF pathways from V1 to different target areas refine with a similar rate. We injected the bidirectional tracer CTb into V1 of juvenile ferrets, and visualized the distribution and pattern of orthogradely labeled axon terminals in extrastriate cortex. We analyzed the refinement of FF terminals in each target area by quantifying the density of labeled synaptic boutons and axonal processes, and interbouton intervals along individual labeled axons. As early as 4 weeks of age, orthogradely labeled FF axon terminals were found in areas 18, 19, 21, and (Suprasylvian cortex) Ssy. Orthogradely labeled axons and retrogradely labeled FB cells were organized into overlapped clusters, indicating reciprocal FF and FB connections of each extrastriate area with V1 (though FB label was always more extensive). Bouton density of FF projections to target areas 18, 19, and 21 declined steadily from 4 to 8 weeks postnatal. However, in area Ssy this decline was delayed somewhat, not occurring until after 6 weeks of age. Mean interbouton intervals along individual FF axons to all visual areas increased over the same postnatal period (6-8 weeks). Similarly, we observed a concomitant moderate decrease in axon density in areas 18, 21, and Ssy during this postnatal period. Thus the decline in bouton density is partly attributable to a reduction in axon density (pruning of supernumerary axon collaterals) as well as a loss of boutons along individual axons (leading to larger interbouton intervals). These data suggest that FF circuits from V1 to its main extrastriate target areas remodel largely synchronously in the weeks following eye opening. Furthermore, both FF and FB cortical circuits share a broadly similar developmental timecourse in the weeks following eye-opening; this suggests that postnatal visual experience is critical for the refinement of both FF and FB cortical circuits. However, the refinement of FF projections from V1 to its targets appears to be delayed 1-2 weeks relative to the refinement of FB projections from those targets to V1.

Disclosures: **R. Khalil:** None. **V.C. Ramirez:** None. **J.B. Levitt:** None.

Poster

678. Sensory Systems Development: Activity and Circuits

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Program#/Poster#: 678.17/D29

Topic: A.08. Development of Motor, Sensory, and Limbic Systems

Title: Developmental changes of spontaneous and sensory evoked activity of the habenular circuits in zebrafish

Authors: *S. L. FORE[1], S. K. JETTI[2], C. V. DIAZ[1], M. HOFFMANN[3], E. BARTOSZEK[1], E. YAKSI[1];
[1]Med., Kavli Inst. for Systems Neurosci. - CNC, Trondheim, Norway; [2]MIT, Cambridge, MA; [3]Charite, Berlin, Germany

Abstract: The Habenula (Hb) is an evolutionary conserved brain structure, subdivided into different nuclei, that is shown to play an important role in associative learning, stress, mood disorders as well as in social interactions such as aggression. Our laboratory has recently highlighted the presence of highly structured spontaneous activity in the habenular neurons. This activity might have a potential role in processing sensory stimuli and comparing this information with the animals' internal behavioral states (such as fear, arousal, stress etc.). The behavioral repertoire of animals evolves and maturates throughout development. For example, it was recently shown that complex social interactions progress during zebrafish development. Similar studies in the past also provide evidence for increased performance at associative learning tasks across different developmental stages in zebrafish.
In this project we aimed to study how spontaneous and sensory driven activity changes throughout development, what mechanisms are underlying these changes and what would be the consequence of these changes with respect to neural computations. In order to achieve this, we measured the activity of habenular neurons at several developmental stages. Our preliminary results suggest a strong change in firing rates of habenular neurons as well as a prominent reorganization in the spatio-temporal patterning of habenular activity. We are currently further investigating transformations in habenular architecture that could underlie these functional changes and how this might link to the changing behavioral repertoire of developing zebrafish.

Disclosures: S.L. Fore: None. **S.K. Jetti:** None. **C.V. Diaz:** None. **M. Hoffmann:** None. **E. Bartoszek:** None. **E. Yaksi:** None.

Poster

678. Sensory Systems Development: Activity and Circuits

Location: Halls B-H

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Program#/Poster#: 678.18/D30

Topic: A.05. Axon and Dendrite Development

Support: JSPS Grant-in-Aid for Challenging Exploratory Research

Title: Roles of neuronal activity in the establishment of neocortical neuron identity.

Authors: *T.-C. WANG, C. HANASHIMA;
Ctr. For Developmental Biology, RIKEN, Kobe / Hyogo, Japan

Abstract: The mammalian neocortex comprises of diverse neurons that can be classified into six major layers according to their gene expression profiles, connectivity, and dendritic patterns. These neurons are generated from progenitor cells that reside in the ventricular zone in a fixed temporal order. Lineage tracing experiments of early cortical progenitors have shown that a single progenitor cell can contribute to neurons of multiple layers [Eckler *et al*., 2015]. Furthermore, reports have also demonstrated that upper-layer neurons can be reprogrammed to deep-layer neuron identity after they migrate into the cortical plate [De la Rossa *et al.*, 2013], raising the possibility that neuronal identity also utilize cues during the post-mitotic stages. In addition to transcriptional regulation, layer IV neurons require thalamocortical inputs for dendritic development and cellular rearrangements, but the relationship between genetic codes and neuronal activity, as well as the critical time window for layer IV neuron specification remain largely elusive. To assess the contribution of intrinsic and extrinsic mechanisms in neocortical neuron development, we inhibited neuronal activity during the early stage of neocortical layer IV neuron differentiation. Our results indicate that the reduction of neuronal excitability affects both the integration and dendritic maturation of layer IV neurons. We further elucidate the crosstalk between intrinsic and extrinsic cues for layer IV neuron maturation.

Disclosures: **T. Wang:** A. Employment/Salary (full or part-time): Center for Developmental Biology, RIKEN. **C. Hanashima:** None.

Poster

678. Sensory Systems Development: Activity and Circuits

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Topic: A.08. Development of Motor, Sensory, and Limbic Systems

Support: General Researcher Program (#2013058415) of National Research Foundation of Korea

Future Systems Healthcare Project of KAIST

Title: Quasi-regular structure of ON and OFF retinal mosaics provides a common organizing principle of functional maps in V1

Authors: *J. JANG[1], C. LEE[1], S.-B. PAIK[1,2];
[1]Dept. of Bio and Brain Engin., [2]Program of Brain and Cognitive Engin., KAIST, Daejeon, Korea, Republic of

Abstract: Primary visual cortex (V1) is organized into functional maps that represent specific features of visual stimulus such as preferred orientation and spatial frequency. Recent studies reported that the structure of a cortical functional map is strongly correlated to the local organization of ON and OFF afferents from the retina and thalamus (Lee et al., 2016; Kremkow et al., 2016). In addition, another study suggested that the geometry of these functional maps in the same cortical area may be correlated to each other, as in the case between the preferred orientation and spatial frequency maps (Nauhaus et al., 2012). Here, we suggest an idea that the quasi-regular structure of ON and OFF retinal ganglion cell (RGC) mosaics seeds various functional maps in V1, in a way that their geometries are systematically correlated to each other as observed in previous studies. Using computer simulations of model retinal mosaics and visual cortex, we could successfully reconstruct the systematic organizations of orientation, direction and spatial frequency maps in V1 with an identical set of ON and OFF RGC mosaics, and could explain geometrical relationships between different maps.
A previous model proposed that orientation map can be seeded by moiré interference between ON and OFF RGC mosaics (Paik & Ringach, 2011). The model assumed that the preferred orientation is determined by the alignment of an ON-OFF dipole—a pair of neighboring ON and OFF RGCs. Based on this model, we showed that the preferred direction and spatial frequency of a V1 neuron can be determined by the direction and size of a dipole. In the moiré interference pattern, we observed the dipole properties - alignment, direction and size - were spatially correlated, and this originated correlation between the functional maps. First, the direction of dipole flips at the center of iso-orientation domain, resulted in the subdivision of the domain into two opposite direction domains as previously reported (Weliky et al., 1996). Next, the spatial gradation of the alignment and the size of dipole were correlated orthogonal to each other in moiré interference, and this induced the perpendicular intersection between the contours of orientation and spatial frequency maps as observed (Nauhaus et al., 2012). These results indicate that the moiré interference of retinal mosaics may seed various functional maps in V1 and correlate them systematically.
Our model, for the first time, offers a common developmental mechanism for the various functional maps in V1 and explains their structural correlations. This suggests that the structure of periphery in sensory system may determine the basic framework of cortical functional circuits**.**

Disclosures: J. Jang: None. **C. Lee:** None. **S. Paik:** None.

Poster

678. Sensory Systems Development: Activity and Circuits

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Topic: A.08. Development of Motor, Sensory, and Limbic Systems

Support: NIH P30-GM-32128

Title: NMDA-dependent competition drives subcellular topography of multisensory inputs in the tadpole tectum

Authors: A. S. HAMODI, 82072, *K. G. PRATT;
Dept. of Zoology and Physiol., Univ. of Wyoming, Laramie, WY

Abstract: The development of topographic maps is known to require both molecular cues and activity-dependent mechanisms. In addition to the topographic map, there is an additional level of subcellular topography involving different sets of axons targeting specific regions of an individual dendrite. However, the mechanism that guides different axonal inputs to different regions of the dendrite is not clear. Here, We address this in the tadpole optic tectum, where tectal neurons receive both visual and non-visual inputs. The visual inputs consistently innervate the distal region of the tectal dendrite, while the non-visual inputs that enter the tectum via the hindbrain (HB) target the proximal region. Field-potential recordings from the tectal neuropil at stage 49 combined with axon imaging studies show that exposing tadpoles to the NMDA receptor antagonist MK 801 (25uM) at the stage when retinal ganglion cell (RGC) and HB axons arrive to the tectum (stage 39) disrupts lamina-specific targeting of RGC and HB axons, whereas blocking action potentials with the sodium channel blocker TTX (1uM) did not. Furthermore, rearing tadpoles in MK 801 after the circuit has formed (stage 45) did not disrupt the normal spatial pattern of RGC and HB inputs along the tectal neuropil. These results indicate that the formation of subcellular dendritic topography of multisensory inputs, but not their maintenance, depends on NMDAR activation. The requirement of NMDAR activation for subcellular topography suggests that this process is competition-dependent. To test this, we disrupted axon competition by monocular enucleation at stage 34. Monocular enucleation experiments revealed that mechanosensory axons are less focused in space and extend into the vacant dendritic region that is normally occupied by RGC axons, thus confirming the role of competition between two sensory inputs for dendritic real estate.

Disclosures: A.S. Hamodi: None. **K.G. Pratt:** None.

Poster

678. Sensory Systems Development: Activity and Circuits

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Program#/Poster#: 678.21/D33

Topic: A.08. Development of Motor, Sensory, and Limbic Systems

Title: The effect of omega-3 fatty acids on murine visual function and plasticity

Authors: *E. CENTOFANTE[1], L. ANEZ-BUSTILLOS[2], M. A. BAKER[2], N. HODGSON[1], T. K. HENSCH[1,3], M. PUDER[2], M. FAGIOLINI[1];
[1]Neurol., [2]Dept. of Surgery and the Vascular Biol. Program, Boston Children's Hosp., Boston, MA; [3]Harvard Univ., Cambridge, MA

Abstract: Omega-3 fatty acids (O3FA), including docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), play a critical role in the maturation and stability of neuronal circuits throughout life stages. O3FA are essential structural components of membranes and serve major functions in synaptic function. Here we investigated how O3FA-enriched diet affects visual system function and its plasticity in mice. Pregnant female mice from C57BL/6 background were fed either O3FA-enriched or standard rodent chow diet starting approximately from embryonic date E10.5 and throughout the lifespan. When compared to 6-8 week-old control mice, O3FA-fed animals showed significantly higher contents of DHA and EPA in brain tissue. Interestingly, EPA is not supplemented in the experimental diets yet significantly higher in the O3FA-fed mice compared to controls. These differences were present even in aging animals (postnatal day (P) 500). No differences were detected across feeding paradigms in the general health and weight of the mice suggesting no detrimental effects of a chronic exposure to an O3FA-enriched diet. Visual evoked potentials (VEP) were then evaluated. VEP were first recorded from anesthetized animals at the peak of the critical period for visual cortex (P24-34). No significant differences were revealed in the response to low spatial frequency stimulus and spatial visual resolution across the two feeding paradigms. The effects of O3FA-enriched diet were assessed on the expression of ocular dominance plasticity by short term monocular deprivation (MD, 4 days) at P24-P28. Surprisingly, O3FA-fed mice did not show a depression in the response of the deprived eye to the low spatial frequency stimuli despite exhibiting a significant loss in spatial acuity. Cortical inhibition mediated by parvalbumin interneurons (PV) enwrapped by specialized extracellular matrix, perineuronal nets (PNN), control the expression of ocular dominance plasticity. Immunohistochemistry analysis revealed a significant decreased in PV intensity after MD in chow- but not in O3FA-fed mice. Visual function was then analyzed in adult (P150-200) and aging (>P500) mice. There was an age-dependent decrease in VEP response in chow-fed mice that was prevented by the O3FA-enriched diet. VEP amplitude was significantly increased in response to all spatial frequencies, resulting in a higher visual acuity in the aging O3FA-fed mice. Interestingly, the PV and PNN circuits in aged mice had a significant increase in PV and PNN intensity in O3FA-fed mice compared to age matched chow-fed mice. Overall, these results suggest a possible neuroprotective effect of O3FA-enriched diet on visual function.

Disclosures: E. Centofante: None. **L. Anez-Bustillos:** None. **M.A. Baker:** None. **N. Hodgson:** None. **T.K. Hensch:** None. **M. Puder:** None. **M. Fagiolini:** None.

Poster

679. Genetic Mechanisms in Autism Models

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 679.01/D34

Topic: A.07. Developmental Disorders

Support: NIH/OD DP5OD009134

NIH/NICHD U54HD083092

Autism Speaks

Title: Loss of autism-associated gene Cntnap2 has broad effects on neuropsychiatric features in juvenile rats

Authors: *D. CONNOLLY, S. VEERARAGAVAN, S. SORIANO, A. J. LIANG, L. YUVA, R. PAYLOR, R. C. SAMACO;
Baylor Col. of Med., Houston, TX

Abstract: Mutations in the *CNTNAP2* gene have been associated with a wide spectrum of neurobehavioral outcomes and a number of neuropsychiatric disorders. Behavioral phenotypes have been characterized in *Cntnap2*-NULL mice, but the complete loss of CNTNAP2 in mice may not fully model the consequences of heterozygous deficiency of the gene as observed in humans. Disease-related features reported in adult mice raise the question of whether similar findings would manifest during early stages of life. In addition, sex-specific differences due to the loss of *Cntnap2* in rodents are poorly understood. Given the availability of a novel *Cntnap2* loss-of-function rat model, we set out to characterize behavioral phenotypes in juvenile male and female rats with either the loss of one (HET) or both (NULL) copies of *Cntnap2*. We found that juvenile *Cntnap2*-HET and -NULL rats display multiple neurobehavioral deficits, including increased obsessive compulsive-like behaviors, increased play behavior, hyperactivity, and an increased acoustic startle response. In some cases, behavioral impairments appeared to manifest only in rats that completely lacked CNTNAP2. Furthermore, sex-specific differences were observed only in the number of ultrasonic vocalizations emitted during play behavior. In comparison with reported findings in the *Cntnap2* mouse model, our study indicates that *Cntnap2* deficiency in the rat results in different behavioral outcomes with the exception of hyperactivity. Taken together, these findings provide insight into the consequences of *Cntnap2* deficiency in a complementary, evolutionarily divergent rodent species and suggest common *Cntnap2*-mediated behavioral phenotypes among genetic rodent models may be useful behavioral outcome measures in preclinical studies.

Disclosures: **D. Connolly:** None. **S. Veeraragavan:** None. **S. Soriano:** None. **A.J. Liang:** None. **L. Yuva:** None. **R. Paylor:** None. **R.C. Samaco:** None.

Poster

679. Genetic Mechanisms in Autism Models

Location: Halls B-H

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Topic: A.07. Developmental Disorders

Support: RWJ Harold Amos Award

NIH NS049453-08

Title: TBCK-encephalopathy, a novel syndrome associated with mTOR over inhibition and mitochondrial dysfunction

Authors: ***X. R. ORTIZ-GONZALEZ**, J. A. TINTOS-HERNANDEZ, *D. C. WALLACE; CMEM, Children's Hosp. of Philadelphia, Philadelphia, PA

Abstract: Over-activation of mTOR signaling has been implicated in pediatric neurologic disorders as well as in adult neurodegenerative disease. However, very little is known about the effects of chronic mTOR inhibition in the brain. We have recently discovered a novel pediatric intellectual disability syndrome, TBCK-associated encephalopathy, uniquely characterized by mTOR over-inhibition. Our cohort of patients with homozygous null TBCK mutations present with progressive central and peripheral neurodegenerative features. Intriguingly, they also exhibit mitochondrial dysfunction, including mtDNA (mitochondrial DNA) depletion. Given that TBCK knockdown leads to downregulation of mTORC1, which is known to regulate oxidative phosphorylation, mitochondrial biogenesis, and mitophagy, we hypothesize that TBCK deficiency causes a severe neurodegenerative phenotype due to chronic mTORC1 inhibition resulting in progressive mitochondrial dysfunction. Specifically, our preliminary data show downregulation of mitochondrial biogenesis via PGC1-alpha, decreased mtDNA copy number, and upregulation of mitophagy in *TBCK*$^{-/-}$ fibroblasts. Therefore we propose to further characterize the mitochondrial defects in *TBCK*$^{-/-}$ fibroblasts and the effects of mTOR modulators. We then propose to generate TBCK$^{-/-}$ IPSC-derived neurons and test the effects of mTOR and mitochondrial targeted therapeutic interventions. This work could elucidate a fundamental disease mechanism in neurodegeneration, where mTOR dysregulation and mitochondrial defects are almost invariably found.

Disclosures: **X.R. Ortiz-Gonzalez:** None. **J.A. Tintos-Hernandez:** None. **D.C. Wallace:** None.

Poster

679. Genetic Mechanisms in Autism Models

Location: Halls B-H

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Program#/Poster#: 679.03/E2

Topic: A.07. Developmental Disorders

Title: Haploinsufficiency of CHD8 results in macrocephaly and aberrant neurodevelopmental gene expression in mice

Authors: ***A. L. GOMPERS**[1], L. SU-FEHER[1], T. STRADLEIGH[1], I. ZDILAR[1], R. ASRAFUZZAMAN[2], G. KAUSHIK[2], D. VOGT[3], J. L. R. RUBENSTEIN[3], A. VISEL[4], L. A. PENNACHIO[4], D. DIXEL[4], K. ZARBALIS[2], A. S. NORD[1], A. S. NORD[1];
[1]UC DAVIS, Davis, CA; [2]Shriners Hosp. for Children, Inst. for Pediatric Regenerative Med., Sacramento, CA; [3]Dept. of Psychiatry, Univ. of California, San Francisco, San Francisco, CA; [4]Lawrence Berkeley Natl. Lab., Berkeley, CA

Abstract: Regulation of chromatin structure and DNA packaging plays a critical role in gene expression. Recent exome sequencing of aAutism trios has revealed that coding mutations in the gene encoding the chromodomain helicase DNA binding protein protein 8 (CHD8) results in macrocephaly, aberrant craniofacial morphology, mild-to-severe cognitive impairment, seizures, gastrointestinal problems and autism. The macrocephaly phenotype has been recapitulated in zebrafish. However, the molecular mechanisms underlying how loss of CHD8 results in macrocephaly, intellectual disability and autism are unclear. Toward this end, we generated a ChdD8 mutant mouse using the Cas9/CRISPR system at a position 5' of the majority of human mutations identified in autism exome sequencing studies. Similar to previously reported, homozygous deletion of ChdD8 is embryonic lethal in mouse. To uncover how haploinsufficiency of ChdD8 results in aberrant brain development we profiled forebrain gene expression patterns of ChdD8 heterozygotes (ChdD8+/-) and wild-type littermates during neurodevelopment by isolating forebrain at embryonic day (e)12.5, e14.5, e17.5, p0 and adults. We found significant changes in expression of early neurodevelopmental and differentiation genes in ChdD8+/- forebrain, including genes for RNA processing and chromatin organization. We further identified changes in genes that are low in expression early in development and that gradually increase during neuronal differentiation, including genes involved in synaptic transmission that are hallmarks of more mature neurons. Consistent with an altered expression of early neurodevelopmental genes, we found that ChdD8+/- mice have an increase in neuronal

proliferation in the ventricular zone in at the peak of neurogenesis, at e13.5 by performing EdU incorporation. We observed increase at P7 in cortical length and cortical thickness utilizing whole mount imaging and Nissl staining. Cursory examination of cortical lamination reveal no obvious differences in layer specification in at postnatal day 1. Adult ChdD8+/- mice have no difference in interneuron cell types or interneuron counts. This study revealed transcriptional and neurodevelopmental mechanisms by which loss of ChdD8 results in macrocephaly, and suggests significant changes neurogenesis and synaptic processes that may underlie behavioral deficits.

Disclosures: **A.L. Gompers:** None. **L. Su-Feher:** None. **T. Stradleigh:** None. **I. Zdilar:** None. **R. Asrafuzzaman:** None. **G. Kaushik:** None. **D. Vogt:** None. **J.L.R. Rubenstein:** None. **A. Visel:** None. **L.A. Pennachio:** None. **D. Dixel:** None. **K. Zarbalis:** None. **A.S. Nord:** None. **A.S. Nord:** None.

Poster

679. Genetic Mechanisms in Autism Models

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Program#/Poster#: 679.04/E3

Topic: A.07. Developmental Disorders

Support: Simons Foundation (SFARI #342005)

Title: Migration of cortical interneurons is modulated by MTOR signaling in the LgDel model of 22q11.2 DS

Authors: *T. M. MAYNARD[1], E. M. PARONETT[2], D. W. MEECHAN[2];
[1]Pharmacol. and Physiol., [2]GW Inst. for Neurosci., George Washington Univ., Washington, DC

Abstract: Interneurons are essential for the proper function of the neuronal circuitry of the cerebral cortex, and disruptions in interneurons have been identified as a key pathological target in several behavioral disorders, including autism and schizophrenia. We have previously shown in a mouse model of 22q11.2 Deletion Syndrome (22q11.2 DS), a neurodevelopmental syndrome strongly associated with autism and schizophrenia, that the migration of interneurons into the cortex is delayed and disrupted, leading to an aberrant distribution of interneurons in the cortex. This disruption in interneuron migration is due to dysregulated signaling via the Cxcr4 cytokine receptor, which has been shown to act via modulation of the mammalian target of rapamycin (MTOR) signaling pathway in several models of cell motility. We have used a conditional genetic approach to disrupt MTOR signaling in both *LgDel* and WT mice, by using a Dlx5/6-Cre line to specifically ablate either the MTOR-repressor *Tsc2*, or *Mtor* itself, specifically in interneuron precursors. We found that heterozygous loss of *Mtor* appears to amplify the

migration defects normally observed in the *LgDel* cortex, with compound *LgDel*;*Mtor*$^{+/-}$ interneurons showing disorganized distributions; while the compound LgDel;*Tsc2*$^{+/-}$ cortex shows that few interneurons enter the cortex at E14. Thus, genetic complementation reveals that MTOR signaling appears to be disrupted in the *LgDel* cortex. MTOR signaling is itself implicated in behavioral disorders such as autism, and multiple genetic syndromes with autism-like behavioral consequences have been described that involve *Mtor* and its interacting partners. Thus, it is possible that this signaling mechanism may be a point of convergence between 22q11.2 DS and other, genetically distinct forms of syndromic behavioral disorders.

Disclosures: T.M. Maynard: None. **E.M. Paronett:** None. **D.W. Meechan:** None.

Poster

679. Genetic Mechanisms in Autism Models

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Topic: A.07. Developmental Disorders

Support: SIMONS Foundation

Title: Treatment with novel ERK inhibitor rescues ASD pathophysiology in 16p11.2 deletion mouse model.

Authors: *J. PUCILOWSKA[1], C. KELLY[2], J. KARLO[2], A. GOZZI[3], R. BRAMBILLA[4], G. E. LANDRETH[2];
[1]Neurosci., [2]Neurosciences, Case Western Reserve Univ., Cleveland, OH; [3]Inst. Italiano di Tecnologia, Rovereto, Italy; [4]Cardiff Univ., Cardiff, United Kingdom

Abstract: Autism Spectrum Disorders (ASDs) are complex, highly heritable neurodevelopmental disorders affecting 1 in 100 children. Our research delineates the contribution of the ERK MAP kinase pathway to the pathogenesis of autism associated with copy number variation (CNV) of human chromosomal region *16p11.2*. CNVs of the *16p11.2* region are genetically linked to 1% of all ASDs. The *16p11.2* locus contains 27 genes, including the ERK1 gene (*MAPK3*). Mutations in upstream elements regulating the ERK pathway are genetically linked to autism and other disorders of cognition including the neuro-cardio-facial cutaneous syndromes (NCFC). ERK1 and its homolog ERK2 are central elements of the MAP kinase pathway governing neural development and synaptic plasticity. We provide direct evidence connecting the ERK kinases to the developmental abnormalities and behavioral deficits observed in the 16p11.2del mouse and show that treatment with novel ERK inhibitor rescues the aberrant pathophysiology in these mice.

We report that the *16p11.2del* murine model exhibits a reduction in brain and body size and perturbations in cortical cytoarchitecture similar to those observed in ERK KO mice. Importantly, we observed a paradoxical increase in ERK signaling in the *16p11.2del* mice, which is coincident with the development of aberrant cortical cytoarchitecture. The *16p11.2del* mice exhibit many abnormal behaviors including anxiety, hyperactivity and impaired memory. Since the aberrant ERK upregulation may be amenable to pharmacological intervention, we treated the *16p11.2del* mice with a novel, brain permeant ERK inhibitor. We employed two treatment paradigms: 1) prenatal, where pregnant dames were treated for 5 consecutive days starting at E10.5; 2) adult, where adult mice were treated for 5 consecutive days starting at P90. We report that prenatal treatment with the ERK inhibitor rescued developmental deficits in neurogenesis and cortical cytoarchitecture as well as aberrant behaviors in the *16p11.2del* mice, whereas adult treated mice showed restoration of some behavioral deficits (e.g. hyperactivity). Importantly, these data suggest that dysregulation of ERK activity can be rescued by two mechanisms, developmentally (through the rescue of cell cycle regulators downstream of ERKs) and in adult mice, presumably due to its synaptic actions.
Our findings provide strong evidence connecting the ERK MAP kinases to the developmental abnormalities in the *16p11.2* deletion. Based on the reversal of the anatomical and behavioral deficits in the *16p11.2del* mouse model, we suggest that treatment with ERK inhibitors can lead to therapeutic interventions in patients with 16p11.2 CNVs.

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Poster

679. Genetic Mechanisms in Autism Models

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Topic: A.07. Developmental Disorders

Support: NSFC Grant 91232303

Title: Medial prefrontal cortex microcircuit dysfunction in NL3 R451C knock-in mice

Authors: ***W. CAO**[1], Y.-L. DU[1], Q.-Q. XIA[1], S. LIN[1], Q. YANG[1], J. XIA[2], J.-Y. XU[1], J.-H. LUO[1];
[1]Sch. of Basic Med. Sci., Zhejiang Univ., Zhejiang, China; [2]Div. of Life Sci. and State Key Lab. of Mol. Neurosci., The Hong Kong Univ. of Sci. and Technol., Hong Kong, China

Abstract: Neuroligins (NLs) are postsynaptic cell adhesion molecules that are related with autism spectrum disorders (ASDs). The neuroligin-3 (NL3) amino acid substitution (R451C) mutation was found in two brothers with ASDs in a Swedish family, and caused social novelty deficits in the specific mutation knock-in mice. The medial prefrontal cortex (mPFC), a brain region that is closely associated with neuropsychiatric disorders including autism. However, little is known about the roles of NL3 during the development of glutamatergic and GABAergic circuitry in mPFC, particularly the roles of NL3 that associated with fast-spiking (FS) interneurons. Here, our electrophysiology data demonstrated decreased N-methyl-D-aspartate receptors (NMDA receptors) function of pyramidal neurons and declined excitability of fast-spiking (FS) interneurons in NL3 R451C knock-in (KI) mice. Additionally, the development and maturity of GABAergic synapses on pyramidal neurons and glutamatergic synapses on FS cells were abnormal in NL3 R451C KI mice compared with WT mice. Together, our findings suggest that the mPFC microcircuit dysfunction may contribute to the ASD-like phenotypes in NL3 R451C KI mice.

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Poster

679. Genetic Mechanisms in Autism Models

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Topic: A.07. Developmental Disorders

Support: AMED Brain/MINDS

JSPS Grant-in-Aid for Scientific Research(C)

NPO Rett Syndrome Supporting Organization

Title: Generation and analysis of Rett syndrome model marmoset

Authors: ***N. KISHI**[1,2], K. SATO[3], M. OKUNO[1], T. ITOU[1], H. J. OKANO[4], E. SASAKI[3], H. OKANO[1];
[1]RIKEN BSI, Saitama, Japan; [2]Dept of Physiol., Keio Univ. Sch. of Meidicine, Tokyo, Japan; [3]CIEA, Kawasaki, Japan; [4]Jikei Univ. Sch. of Med., Tokyo, Japan

Abstract: In the human brain, there are two major functional domains. One has been conserved in all mammals through evolution and governs fundamental functions such as reward, emotion and memory; the other is unique to primates, and is acquired through the enlargement of the

cerebral cortex governing special functions such as tool use, language, and self-consciousness. Thus, to properly understand these brain functions, we need appropriate animal models for studying each function. Animal models that are used to analyze brain functions are different in each case. In the former, a reductive approach is adopted based on gene manipulation using models such as genetically-modified fish and rodents, while in the latter, the main approach is psychological and involves complex behavior analysis using non-human primates such as macaque monkeys. Many researchers believed that the complementary nature of genetic engineering technologies in rodent and fish models and cognitive neuroscience techniques in primate research would lead to progress in this research field. However, due to lack of appropriate animal models that can be analyzed in both aspects of the brain's functions, contact points between these two approaches have been limited.
The development of genetically engineered non-human primates has attracted attention for its potential to connect the two research fields. Recently, we succeeded in creating the world's first transgenic primate using marmosets. This technological breakthrough provides a potential paradigm shift by enabling researchers to analyze both the brain functional domains using various model marmosets.
Currently, we are developing a technique for creating knockout marmosets using zinc finger nuclease (ZFN) technology. By combining this technique with the development of cognitive information for marmoset brain analysis, innovative MRI imaging technology and marmoset genetic analysis tools, we created and are analyzing MECP2 mutant marmosets suitable for research on Rett syndrome. MRI imaging shows that the brain size of MECP2 +/- marmoset is smaller than wild-type one by approximately 10% at 18 months of age. Use of MECP2 deficient marmoset will not only reveal pathogenesis of Rett syndrome, but also potentially contribute to future therapeutic strategies for Rett syndrome.

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Poster

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Topic: A.07. Developmental Disorders

Support: MIND Institute IDDRC NIH Grant U54 HD079125

Title: Initial characterization of a new loss-of-function mouse model of the autism susceptibility gene Chd8

Authors: *M. PRIDE[1], I. ZDILAR[1], A. GOMPERS[1], A. VISEL[2], L. PENNACCHIO[2], D. DICKEL[2], J. L. SILVERMAN[1], J. N. CRAWLEY[1], A. S. NORD[1];
[1]UC Davis, Sacramento, CA; [2]Lawrence Berkeley Natl. Lab., Berkeley, CA

Abstract: Autism spectrum disorder (ASD) is a heterogeneous neurodevelopmental disorder in which it may be possible to stratify subtypes based on genetic mutations and behavioral profiles. Gene-based subtype identification recently discovered the chromodomain helicase DNA-binding protein 8 (*Chd8*) as a likely candidate for a specific subtype of ASD (Bernier et al., 2014, O'Roak et al., 2012, Talkowski et al., 2012 and Neale et al., 2012) with associative cognitive impairments, seen in approximately 60% of human cases with mutations in *Chd8* (Bernier et al. 2014). Genetic engineering via the Cas9/CRISPR system was employed to generate a constitutive *Chd8* mutant mouse model. Briefly, synthetic guide RNA was injected along with Cas9 mRNA to mouse oocytes, and F0s carrying mutations were genotyped and bred to expand lines that harbored a mutation. We selected a line that harbors a short deletion in the fifth exon of *Chd8* causing a frameshift resulting in a predicted loss-of-function allele. We evaluated this new line of mice with a constitutive mutation in *Chd8* on a sequence of behavioral assays relevant to the diagnostic and associated symptoms of autism. Cognitive deficits were detected in the *Chd8* heterozygotes on two distinct learning and memory tasks, novel object recognition and fear conditioning. Social scores were normal on both 3-chambered social approach and male-female reciprocal social interactions with ultrasonic vocalizations. No spontaneous motor stereotypies, repetitive self-grooming or unusual levels of marble burying were detected in either genotype. Other behavioral assays conducted included two anxiety-related tasks, elevated plus-maze and light↔dark transitions, open field exploration, hot plate nociception, acoustic startle and prepulse inhibition, and measures of general health. Our behavioral impairments suggest loss of *Chd8* is a contributing factor to the cognitive deficits seen clinically. These findings highlight the need to further examine this unique *Chd8* mouse model with extensive pathophysiology and behavioral phenotyping efforts.

Disclosures: M. Pride: None. **I. Zdilar:** None. **A. Gompers:** None. **A. Visel:** None. **L. Pennacchio:** None. **D. Dickel:** None. **J.L. Silverman:** None. **J.N. Crawley:** None. **A.S. Nord:** None.

Poster

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Support: NSF-GRFP DGE-0707424

NIH/NIMH R01 MH081754-02R

Pilot Grant from UCLA CART

Title: Synaptic and network abnormalities in the Cntnap2 mouse model of autism

Authors: *M. T. LAZARO[1,3,4], O. PEÑAGARIKANO[5], I. BACHMUTSKY[5], T. IKRAR[2], R. SANTOS[2], A. MYLAVARAPU[5], S. CHANDRA[5], H. DONG[4], X. XU[2], D. H. GESCHWIND[4,6,7], P. GOLSHANI[7,8];
[1]Neurosci., Univ. of California, Los Angeles, CA; [2]Univ. of California, Irvine, CA; [3]Neurosci. Interdepartmental Program, [4]Program in Neurogenetics, Dept. of Neurology, David Geffen Sch. of Med., [5]Program in Neurogenetics, [6]Ctr. for Autism Res. and Treatment, Semel Institute, David Geffen Sch. of Med., [7]Dept. of Neurol., UCLA, Los Angeles, CA; [8]West Los Angeles VA Med. Ctr., Los Angeles, CA

Abstract: Recessive truncating mutations in CNTNAP2 cause Cortical Dysplasia Focal Epilepsy (CDFE), a syndrome that is highly co-morbid with Autism Spectrum Disorder (ASD). Cntnap2/CASPR2 knock-out (KO) mice recapitulate core ASD deficits, including impaired communication and social interactions, as well as repetitive behaviors. KO mice also display seizures, decreased neuronal synchronization and neuronal migration abnormalities (Peñagarikano et al. 2011, Cell). To uncover the mechanisms underlying these phenotypes, we tested whether loss of Cntnap2 results in altered neuronal excitability, synaptic transmission, and microcircuit connectivity. We performed whole-cell in vitro slice recordings from L2/3 pyramidal (Pyr) and parvalbumin (PV) inhibitory neurons of the medial prefrontal cortex (mPFC), an area important for social behavior. Surprisingly, intrinsic neuronal excitability of both cell types was not significantly different between wild-type (WT) and KO mice. We then tested whether loss of Cntnap2 affected synaptic neurotransmission by quantifying miniature excitatory and inhibitory postsynaptic currents (mEPSCs and mIPSCs, respectively) and found a two-fold decrease in mEPSC frequency on Pyr but not PV cells, and no significant change in amplitude for any of these measures. Moreover, cortical input mapping using laser scanning photostimulation (LSPS) with glutamate uncaging further revealed a drastic decrease in both excitatory and inhibitory functional synaptic connectivity. Paired recordings from connected excitatory and PV neurons also point toward a decrease in the size of unitary evoked inhibitory currents. We also performed anatomical studies which show that, while dendritic complexity is normal in Pyr KO neurons, there is a 20% decrease in spine density. Therefore, loss of Cntnap2 affects local synaptic inputs and microcircuit connectivity in the murine mPFC and sheds light on a potential target for therapeutic interventions in the treatment of ASD.

Disclosures: **M.T. Lazaro:** None. **O. Peñagarikano:** None. **I. Bachmutsky:** None. **T. Ikrar:** None. **R. Santos:** None. **A. Mylavarapu:** None. **S. Chandra:** None. **H. Dong:** None. **X. Xu:** None. **D.H. Geschwind:** None. **P. Golshani:** None.

Poster

679. Genetic Mechanisms in Autism Models

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Support: CIHR

Brain Canada

Ontario Brain Institute

Scottish Rite Charitable Foundation

Title: Characterization of TAO2 KO mice as an autism spectrum disorder model and the pathophysiological effects of TAO2 human genetic variants

Authors: *N. MURTAZA[1], M. RICHTER[2], S. WHITE[1], R. SCHARRENBERG[2], F. MORELLINI[2], A. NAUMANN[2], B. SCHWANKE[2], V. KWAN[1], S. WALKER[3], R. YUEN[3], S. W. SCHERER[3], F. CALDERON DE ANDA[2], K. SINGH[1];
[1]Stem cell and cancer research institute, McMaster Univ., Hamilton, ON, Canada; [2]Ctr. for Mol. Neurobio., Hamburg, Germany; [3]The hospital for sick children, Toronto, ON, Canada

Abstract: Atypical brain connectivity is a major factor in the pathophysiology of Autism Spectrum Disorders (ASDs). To model ASD we are studying the thousand and one amino acid kinase 2 (TAO2), known to play a role in neuron development and identified as part of a common ASD CNV region, 16p11.2. Initial studies reveal impairments in social interaction, a common finding in ASD models, and the presence of associated behavioural changes, including altered anxiety levels, impaired spatial and working memory, and alterations in contextual fear conditioning. These mice also have reduced basal level excitatory synaptic activity, corroborated by reduced dendritic arborisation and decreased dendritic spine numbers in the prefrontal cortex. Concurrently, whole genome sequencing of ASD families identified multiple *de novo* and rare-inherited variants in *TAO2*. Expression of the *de novo* variants in cortical neurons revealed altered dendrite and spine morphology, and abnormal activation of known downstream signalling pathways. The identification of multiple functionally validated *de novo* mutations in *TAO2* highlights the significant impact of disruptions in *TAO2.* Further studies will aim to delineate targetable pathways downstream of TAO2 and their role in atypical brain connectivity.

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Poster

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Topic: A.07. Developmental Disorders

Title: Impaired hippocampus-dependent learning and synaptic plasticity in a novel animal model of autism spectrum disorders with telomerase reverse transcriptase overexpression

Authors: *K. PARK;
Biol. Sci., Konkuk Univ., Seoul, Korea, Republic of

Abstract: Recently, we have reported that animals with telomerase reverse transcriptase (TERT) overexpression exhibit reduced social interaction, decreased preference for novel social interaction and poor nest-building behaviors—symptoms that mirror those observed in autism spectrum disorders (ASD). Overexpression of TERT also alters the excitatory/inhibitory (E/I) ratio in the medial prefrontal cortex. However, the effects of TERT overexpression on hippocampal-dependent learning and synaptic efficacy have not been investigated. In the present study, we employed electrophysiological approach in combination with behavioral analysis to analyze hippocampal function of TERT transgenic (TERT-tg) mice in comparison to that of FVB controls. We found that TERT overexpression results in enhanced excitation with no changes in inhibition in the hippocampus and significantly impairs long-term synaptic plasticity. In addition, TERT-tg mice showed poor performance on the Morris water maze test, indicating disrupted hippocampal-dependent spatial learning. Interestingly, the expression levels of phosphorylated CREB and phosphorylated CaMKIIα were significantly decreased while the expression level of CaMKIIα was slightly increased in the hippocampus of TERT-overexpressing group. Our observations highlight the importance of TERT in normal synaptic function and behavior and provide additional information on a novel animal model of ASD associated with TERT overexpression.

Disclosures: K. Park: None.

Poster

679. Genetic Mechanisms in Autism Models

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 679.12/E11

Topic: A.07. Developmental Disorders

Support: SFARI (SVD)

Dup15q Alliance (SVD & JLS)

MIND Institute (JLS)

Intellectual and Developmental Disabilities Research Center (IDDRC) HD079125-01 (JLS)

Ontario Brain Institute (JE & JL)

Title: Neuroanatomical phenotypes in a novel inducible mouse model of Ube3a overexpression and chromosome 15q11.2-13 duplications

Authors: *J. L. SILVERMAN[1], N. BUSCHER[1], N. A. COPPING[1], S. CHRISTIAN[2], J. LERCH[3], S. V. DINDOT[2], J. ELLEGOOD[3];
[1]UC Davis Sch. of Med., Sacramento, CA; [2]Texas A&M, College Station, TX; [3]Mouse Imaging Ctr., Toronto, ON, Canada

Abstract: Maternally derived duplications or triplications of 15q11.2-q13 (Dup15q) are one of the most common genetic variations associated with autism spectrum disorder (ASD), detected in ~1-3% of cases. Overexpression of the ubiquitin protein ligase E3A (*UBE3A*) gene is believed to cause the symptoms observed in Dup15q, as there is a known role of *UBE3A* mutations or deletions as the causal determinant of Angelman Syndrome (AS). UBE3A expresses three isoforms with distinct amino terminal ends that exhibit differential localization and functions in neurons. Our initial neuroanatomical characterization studies focused on mouse *Ube3a* isoform 2 (*Ube3a2*), as it is highly conserved and has been shown to selectively regulate dendritic morphogenesis. An inducible (Tetracycline-Off) transgenic mouse model of *Ube3a2* (TRE-Ube3a2) was generated and crossed to the CamKIIa-tTA transgenic mouse model (Jackson Laboratory #003010), which induces expression of the TRE-transgene in excitatory neurons in the forebrain. We hypothesized that neuronal forebrain overexpression of *Ube3a2* would cause structural neuroanatomical phenotypes compared to wildtype littermate sex and aged matched controls, since neuroimaging in Dup15q patients indicated large hippocampal heterotopias and dysplasias (Boronat et al., 2015). Whole brains were harvested from the behaviorally tested mice, and structural MRI was performed to identify volume changes in different brain regions. MRI revealed that the total brain volume was decreased by (5.0%, $q<0.001$) in the TRE-*Ube3a2*-

tTA mice compared to WT. Localized decreases in absolute volume (mm^3) were found throughout the cortex, with the overall cortex decreasing by (3.8%, $q<0.01$); however the main effect of the TRE-*Ube3a2*-tTA mutation appeared to be in the subcortical gray and white matter. The amygdala ($q<0.001$), hippocampus ($q=0.001$), and striatum ($q<0.0001$) were all significantly smaller in the TRE-*Ube3a2*-tTA mice and the cerebral white matter as a whole was 7.3% smaller ($q<0.0001$) and all major white matter structures were affected. The cerebellum, on the other hand, was the one area in the brain that was largely unaffected by the TRE-*Ube3a2*-tTA mutation. To date, there has been no neuroanatomical analysis of preclinical models that overexpress *Ube3a* in an isoform specific manner. This information is essential to understand how dysregulated expression of UBE3A in the brain results in multiple neurodevelopmental disorders, such as Dup15q, AS and ASD.

Disclosures: J.L. Silverman: None. **N. Buscher:** None. **N.A. Copping:** None. **S. Christian:** None. **J. Lerch:** None. **S.V. Dindot:** None. **J. Ellegood:** None.

Poster

679. Genetic Mechanisms in Autism Models

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Topic: A.07. Developmental Disorders

Support: Division of Intramural Research Program, National Institute of Mental Health, National Institutes of Health, Bethesda, MD.

Self Regional Healthcare Collaborative. Grant #060

Autism Science Foundation

Phelan-McDermid Syndrome Foundation

Title: Identifying potentially haploinsufficient genes in 22q13 deletion syndrome

Authors: *A. R. MITZ[1], A. SHCHEGLOVITOV[2], L. BOCCUTO[3,4], A. THURM[5];
[1]Lab. of Neuropsychology, NIH, Bethesda, MD; [2]Dept. of Neurobio. & Anat., Univ. of Utah, Salt Lake City, UT; [3]JC Self Res. Inst. of the Greenwood Genet. Ctr., Greenwood, SC; [4]Clemson Univ. Sch. of Hlth. Res., Clemson, SC; [5]Pediatrics and Developmental Neuropsychiatry Br., Natl. Inst. of Mental Hlth., Bethesda, MD

Abstract: Large-scale GWAS studies of CNVs or SNPs have been valuable tools for studying mutation load associated with major neuropsychiatric disease (e.g., schizophrenia, autism).

However, haploinsufficiency from gene loss, not mutation, is usually the primary mechanism in chromosomal deletion syndromes. Multiple methods for predicting haploinsufficiency have been reported. Cody et al. (Am J Med Genet C Semin Med Genet, 2015) used gene dosage estimation based on published studies of specific genes. Other groups have developed computational approaches that combine gene co-expression and genetic variation estimates (Huang et al., PLoS Genet, 2010; Petrovski et al., PLoS Genet, 2013; Steinberg et al., Nucleic Acids Res, 2015). We used these two approaches to identify potentially haploinsufficient genes in 22q13 region deleted in patients with 22q13 deletion syndrome (Phelan-McDermid syndrome). 22q13 deletion syndrome is characterized by intellectual disability and paucity of speech, but patients manifest many problems from the involvement of 100 or more genes, depending upon the size and location of the deletion. Clinical cases of this syndrome occur independently of any one locus, ruling out the possibility of a single gene cause. There are also too few clinical cases to otherwise assess the impact of any single deleted gene without confounds of nearby genes implicated in CNS pathology (Sarasua et al., Hum Genet, 2014). To uncover evidence for individual gene haploinsufficiency, gene dosage estimates were compiled for the distal 100 genes in 22q13 through literature review, and dosage predictions were taken from the published algorithm-based haploinsufficiency scores. Gene expression was measured using quantitative real-time PCR in cultured iPSC neurons derived from patients. Based on gene dosage from the literature, 6% (6/100) genes were identified as potentially haploinsufficient and 3% (3/100) were judged conditionally haploinsufficient. However, over half of the genes (55/100) lacked sufficient information to make a determination. Although the *SHANK3* gene is most commonly associated with 22q13 deletion syndrome, the algorithm-based search for potentially haploinsufficient genes identified at least 18 other genes with a higher likelihood for haploinsufficiency. We tested the expression of potentially haploinsufficient genes in iPSC-derived neurons from patients. Our results indicate that several genes in the 22q13 region show evidence for contributing to the clinical features seen in these patients.

Disclosures: **A.R. Mitz:** None. **A. Shcheglovitov:** None. **L. Boccuto:** None. **A. Thurm:** None.

Poster

679. Genetic Mechanisms in Autism Models

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Topic: A.07. Developmental Disorders

Support: MIND Institute's Intellectual and Developmental Disabilities Research Center (IDDRC) Grant HD079125-01 (DJS & JLS)

MIND Institute (JLS)

Title: Generation and preliminary characterization of artificial transcription factors (ATFs) for the regulation of Shank3 in a preclinical model of Phelan McDermid Syndrome and Autism

Authors: C. REN[1], B. R. PYLES[1], M. C. PRIDE[2], N. BUSHCER[2], N. A. COPPING[2], H. O'GEEN[1], *M. YANG[2], D. J. SEGAL[1], J. L. SILVERMAN[2];
[1]MIND Institute, Dept. of Biochem. and Mol. Med., UC Davis Sch. of Med., Davis, CA; [2]MIND Institute, Dept. of Psychiatry and Behavioral Sci., UC Davis Sch. of Med., Sacramento, CA

Abstract: Mutations in SHANK have been observed in ~1% of individuals with ASD. Mouse models with targeted mutations in *Shank3* exhibit well-characterized, reproducible, ASD-relevant behavioral phenotypes. Manipulating gene expression in rodent models is critical to understanding gene functions. We developed an artificial transcription factor (ATF) protein that can be injected intraperitoneally or subcutaneously, crossed the blood-brain barrier, and increased expression of endogenous genes, such as *Ube3a* (Bailus et al., 2016). Herein, animals were given doses of (2mg/ inj., 3x weekly, s.c.) of full length ATF protein designed, cloned and purified to be specific to *Shank3,* for two weeks. Minimal to no toxicity stress was observed with this dosing regimen. Minimal to no toxicity stress was observed with this dosing regimen. RT PCR for RNA and Westerns protein analysis of Shank3 in the brain tissue showed elevated expression levels. Following dose optimization, we performed a custom designed series of assays aimed to assess cognitive dysfunction using simple and complex learning tasks, as previously described (Silverman et al., 2010). We utilized the innovative, operant touchscreen system to evaluate complex, hippocampal and cortical-dependent learning and memory, as well as behavioral flexibility (Silverman et al., 2015; Leach et al., 2016). In addition, we measured endpoints of motor abilities and ASD relevant behavioral phenotypes, such as, locomotion in an open field, balance beam walking and repetitive motor behaviors. Improvements in motor activity, cognitive function or ASD-relevant behavioral phenotypes have important translational value. Phelan McDermid Syndrome, the genetic disorder caused by terminal deletions in 22q13.3 (a region that encompasses the *SHANK3* gene), is associated with a number of motor problems, including generalized developmental delay, neonatal hypotonia, low energy and muscle/motor weakness --- symptoms not commonly found in ASD cases unrelated to *SHANK3* mutations (Phelan and McDermid, 2012, Harony-Nicolas et al., 2015; Mieses et al., 2016.). Our data corroborate the *Shank3B* mouse model (Peca et al., 2011) by recapitulation of motor symptoms and cognitive deficits in PMS. The effect of increased *Shank3* expression by ATF on behavioral outcomes adds to our knowledge of *Shank3* biology, while demonstrating the utility of ATFs as a novel tool for neurodevelopmental disorders.

Disclosures: **C. Ren:** None. **B.R. Pyles:** None. **M.C. Pride:** None. **N. Bushcer:** None. **N.A. Copping:** None. **H. O'Geen:** None. **M. Yang:** None. **D.J. Segal:** None. **J.L. Silverman:** None.

Poster

679. Genetic Mechanisms in Autism Models

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Topic: A.07. Developmental Disorders

Support: SFARI (SVD)

Dup15q Alliance (SVD & JLS)

MIND Institute (JLS)

IDDRC Grant HD079125-01 (JLS)

Title: Selective neuronal forebrain overexpression of Ube3a isoform 2 causes behavioral phenotypes relevant to chromosome 15q11.2-13 duplications

Authors: *N. COPPING[1], S. CHRISTIAN[2], N. BUSCHER[1], M. S. ISLAM[1], M. C. PRIDE[1], J. M. LASALLE[1], S. V. DINDOT[2], J. L. SILVERMAN[1];
[1]UC Davis, Sch. of Med., Sacramento, CA; [2]Texas A&M Univ., College Station, TX

Abstract: Maternally derived duplications or triplications of 15q11.2-q13 (Dup15q) are one of the most common genetic variations associated with autism spectrum disorder (ASD) detected in ~1-3% of cases. Overexpression of the ubiquitin protein ligase E3A (UBE3A) gene is believed to cause symptoms observed in Dup15q and ASD. UBE3A expresses three isoforms with distinct amino terminal ends that exhibit differential localization in neurons. We hypothesize that the overexpression of a single isoform can alter functional behavioral phenotypes. In this study, we have characterized neuronal forebrain overexpression of Ube3a isoform 2 (*Ube3a2*), to discover if this single isoform is sufficient to cause impairments relevant to Dup15q and ASD. An inducible (Tetracycline-Off) transgenic mouse model of Ube3a2 (TRE-Ube3a2) was generated and crossed to the CamK2a-tTA transgenic mouse model (Jackson Laboratory #003010), which induces expression of the TRE-transgene in excitatory neurons located in the prefrontal cortex and hippocampus. The TRE-*Ube3a2* transgene was expressed in cortex at levels approximately 2.5-fold higher than endogenous Ube3a in CamK2a-tTA/TRE-*Ube3a* (TRE-*Ube3a2*-tTA) double transgenic mice compared to wildtype littermate sex and aged matched controls. A battery of behavioral assays relevant to Dup15q and ASD, as well as numerous control assays to detect confounds in physical health or ability to evaluate complex behaviors were conducted as previously described (Crawley et al., 2007; Silverman et al., 2010, 2012). TRE-*Ube3a*2-tTA offspring showed cytoplasm-specific expression of Ube3a2 in forebrain neurons above level of endogenous Ube3a expression. Robust anxiety-like behaviors were observed in two gold standard conflict anxiety assays. As compared to wildtype, TRE-*Ube3a2*-tTA mice exhibited

high anxiety-like phenotypes. A tertiary anxiety-like measurement, the stress-induced hyperthermia assay, corroborated our findings. ASD-relevant behavioral phenotyping identified normal sociability and elevated repetitive, restricted behavior in TRE-*Ube3a*2-tTA offspring. In this first characterization, of a Ube3a isoform-specific transgenic mouse, we confirmed the genetic construct and detected significant functional outcomes in anxiety-like and repetitive behaviors. However, social behaviors were normal. These studies reveal for the first time a strong functional role for Ube3a2, specifically. Importantly, these data suggest Ube3a2 to be a causative determining factor for at least some of the major phenotypic impairments underlying Dup15q.

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Poster

679. Genetic Mechanisms in Autism Models

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he Program for New Century Excellent Talents in University of China

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Title: Investigating the neuronal function of dock4, a autism/dyslexia related gene

Authors: *D. GUO, Y. PENG, C. LIANG, L. SHI;
Jinan Univ., Guangzhou City, China

Abstract: Rho GTPase regulators are key modulators of actin cytoskeleton, which essentially controls neuronal morphogenesis and synapse plasticity. Dock 4, a guanine nucleotide exchange factor (GEF) that activates Rac1, has recently been found in multiple studies as a risk gene in neurodevelopmental disorders such as autism and dyslexia. Previous studies by others and us using in vitro models showed that Dock4 is important for normal neurite outgrowth and spine morphogenesis, but its in vivo function has not been revealed so far. By generating a knockout mice line of DOCK4, we characterized autism-related behaviors and learning and memory abilities when DOCK4 expression is lacking. In particular, we assessed sociability, stereotype

and repetitive behaviors, anxiety, spatial learning and recognition memory, and DOCK4 KO mice showed some features of ASD-like behaviors. Moreover, neuroanatomical analysis of these mice and molecular mechanism regulated Dock4 were characterized. Results from this work will be presented and discussed.

Disclosures: **D. Guo:** None. **Y. Peng:** None. **C. Liang:** None. **L. Shi:** None.

Poster

679. Genetic Mechanisms in Autism Models

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Pennsylvania Department of Health (SAP # 4100042728)

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The Sumitomo Foundation

Title: The role of hormones in male-specific social and behavioral deficits in the protocadherin 10 (Pcdh10) mouse model of autism

Authors: ***S. L. FERRI**[1], M. R. BRUCE[4], H. C. DOW[2], C. C. ANGELAKOS[1], W. T. O'BRIEN[3], H. SCHOCH[5], S. HIRANO[6], R. T. SCHULTZ[7], E. S. BRODKIN[2], T. ABEL[1]; [1]Biol., [2]Psychiatry, [3]Neurosci., Univ. of Pennsylvania, Philadelphia, PA; [4]Temple Univ., Philadelphia, PA; [5]Neurobio. and Behavior, Univ. of California, Irvine, Irvine, CA; [6]Cell Biol., Kansai Med. Univ., Osaka, Japan; [7]Ctr. for Autism Res., Children's Hosp. of Philadelphia, Philadelphia, PA

Abstract: Autism spectrum disorder (ASD) is a neurodevelopmental disability that affects nearly five times as many males as females, but the basis of this male bias is unknown. ASD has been linked to a number of genes that encode synaptic proteins, one of which is protocadherin 10 (PCDH10), an activity-dependent cell adhesion molecule expressed primarily in the amygdala

and striatum. Utilizing mice heterozygous for *Pcdh10* (Pcdh10+/- mice), we previously reported male-specific deficits in juvenile social behavior and fear conditioning, as well as changes in the basolateral amygdala including increased dendritic spine density and decreased NMDA receptor expression, as well as decreased BLA gamma band power in response to lateral amygdala stimulation. New preliminary data indicates that Pcdh10+/- mice emit significantly more ultrasonic vocalizations than their wildtype littermates at postnatal day 6 when separated from their mother, a deficit that has been reported in other mouse models of autism. In addition, males but not females lacking one copy of *Pcdh10* exhibit deficits on a high speed rotarod and hypoactivity during the dark phase in their home cages. In order to investigate the sex specificity of the behavioral deficits, we gonadectomized male mice prior to puberty and found that gonadectomy increased social approach behavior to wildtype levels in the Pcdh10+/- males but did not affect the performance of the wildtype males. Future studies will investigate the effect of gonadectomy on other male-specific behavioral deficits, adult testosterone levels, and the role of neonatal versus pubertal hormones.

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Poster

679. Genetic Mechanisms in Autism Models

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Topic: A.07. Developmental Disorders

Support: SFARI (SVD)

Dup15q Alliance (SVD & JLS)

MIND Institute (JLS)

MIND Institute's Intellectual and Developmental Disabilities Research Center (IDDRC) Grant HD079125-01 (JLS)

Title: Cognitive phenotypes in a novel model of chromosome 15q11.2-13 duplication

Authors: *N. BUSCHER[1], S. CHRISTIAN[2], N. A. COPPING[1], D. RITTER[2,1], S. V. DINDOT[2], J. L. SILVERMAN[1];
[1]UC Davis, Sacramento, CA; [2]Texas A&M Univ., College Station, TX

Abstract: Maternally derived duplications or triplications of 15q11.2-q13 (Dup15q) are one of the most common genetic variations associated with autism spectrum disorder, detected in ~1-3% of cases. Overexpression of the ubiquitin protein ligase E3A (*UBE3A*) gene, which is located at the telomeric end of the 15q11.2-q13 region, is believed to cause the symptoms observed in Dup15q. *Ube3a* expresses three isoforms with distinct amino terminal ends that exhibit differential localization in the cell, and that display different functions in neurons. The initial characterization studies focused on *Ube3a* mouse isoform 2 (*Ube3a2*), as it is conserved among vertebrate species and has been shown to regulate dendritic morphogenesis in pyramidal neurons (Miao et al., 2013). We hypothesized that overexpression of *Ube3a2* disrupts cognitive and behavioral phenotypes. An inducible (Tetracycline-Off) transgenic mouse model of *Ube3a2* (TRE-*Ube3a2*) was generated and crossed to the CamK2a-tTA transgenic mouse model, which induces expression of the TRE-transgene in excitatory neurons located in the prefrontal cortex and hippocampus. The TRE-*Ube3a2* transgene was expressed in cortex at levels approximately 2.5-fold higher than endogenous *Ube3a* in CamK2a-tTA/TRE-*Ube3a* (TRE-*Ube3a2*-tTA) double transgenic mice. Expression of the transgene was limited to excitatory neurons in the cortex and hippocampus and responsive to doxycycline. Breeding pairs of TRE-*Ube3a2* transgenic mice were donated by the Dindot laboratory (Texas A&M). Breeding pairs of B6;CBA-Tg(Camk2a-tTA)1Mmay/J (tTA) were purchased from the Jackson laboratory (#003010). PCR genotyping and Western blotting were done to confirm TRE-*Ube3a2*-tTA overexpression. We designed a custom series of assays aimed to assess cognitive dysfunction using simple and complex learning tasks as previously described (Silverman et al., 2010). Traditional novel object recognition and pavlovian context and cued conditioning were tested. To corroborate and extend, we utilized the innovative operant touchscreen system, evaluating complex, hippocampal and cortical-dependent learning and memory (Silverman et al., 2010, 2015). TRE-*Ube3a2*-tTA subjects exhibited learning and memory impairments in contextual and cued fear conditioning but did not show deficits in novel object recognition, compared to wildtype age and sex matched littermates. In addition, our study measured latencies to criterion in the visual touchscreen based assays. These studies are impactful because they are the first to show that *Ube3a* isoforms play a substantial role in cognitive function.

Disclosures: **N. Buscher:** None. **S. Christian:** None. **N.A. Copping:** None. **D. Ritter:** None. **S.V. Dindot:** None. **J.L. Silverman:** None.

Poster

679. Genetic Mechanisms in Autism Models

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Program#/Poster#: 679.19/E18

Topic: E.02. Cerebellum

Support: European Union grant Human Brain Project HBP-604102

Title: Hyper-excitability and hyper-plasticity in the cerebellar network of IB2 KO mouse model of autism

Authors: *L. MAPELLI[1], T. SODA[1,2], F. LOCATELLI[1], L. BOTTA[3], M. GOLDFARB[4], F. PRESTORI[1], E. D'ANGELO[1,5];
[1]Dept of Brain and Behavioral Sci., Univ. of Pavia, Pavia, Italy; [2]Museo Storico della Fisica e Ctr. Studi e Ricerche Enrico Fermi, Rome, Italy; [3]Dept of Biol. and Biotech. "L. Spallanzani", Univ. of Pavia, Pavia, Italy; [4]Dept of Biol. Sciences, Hunter Col., New York, NY; [5]Brain Connectivity Center, C. Mondino Natl. Neurolog. Inst., Pavia, Italy

Abstract: Autism is a pervasive neurodevelopmental disorder characterized by abnormal social behavior and communication and by various cognitive abnormalities, including hyper-focusing on specific brain processes and difficulties in attention switching and redirection. While marked heterogeneity characterizes autism landmarks both in human patients and in mouse models of the disease, a consistent involvement of the cerebellum has been highlighted. In order to address the cerebellar microcircuit dysfunction in autism pathophysiology, we have analyzed single-neuron and microcircuit activity in the cerebellar cortex of IB2 knock-out (KO) mice (Giza et al., 2010), which carry the same mutation revealed in the familial form of autism known as Phelan-McDermid syndrome. By using patch-clamp electrophysiology and voltage-sensitive dye imaging (VSDi) we observed severe alterations in neural processing in the granular layer microcircuit of IB2 KO mice compared to age and background strain matched wild-type mice. Granule cells showed increased NMDA receptor-mediated currents and intrinsic excitability, while GABAergic inhibition was reduced, bringing about a raise in the excitatory/inhibitory balance (E/I ratio, WT 1.02±0.13 vs KO 1.44±0.13, n=4 for both, $p<0.05$). Moreover, the magnitude of long-term potentiation was almost quadrupled (LTP; WT 35.64±10.64% n=13 vs KO 120.59±49.27% n=12; $p<0.05$) and it spatial extent triplicated (LTP area KO/WT 3.1±0.3, both n=6, $p<0.003$). Both in IB2 KO and in WT mice, the granular layer response was organized in center-surround. Interestingly, IB2 KO mice responses showed larger centers of excitation (29.5±4.9μm vs. 12.9±1.7μm in WT, both n=5, $p<0.01$) and thinner inhibitory surrounds, demonstrating the shift from a "mexican-hat" to a "stove-pipe" profile predicted by Casanova (2003, 2006) on theoretical grounds. Taken together, our results reveal a critical damage in granular layer microcircuit functions that could seriously alter the signals transmitted to the cerebral cortex, thereby altering functions like attention switching and thought coordination, that are believed to be regulated by the cerebellum, and contributing to the pathogenesis of autistic symptoms.

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Poster

680. Physiological Mechanisms in Autism and Autism Models

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Program#/Poster#: 680.01/E19

Topic: A.07. Developmental Disorders

Support: SFARI Explorer Award: 362242

Title: Autism-associated missense mutations in SCN2A impair $Na_V1.2$ sodium channel function

Authors: *R. BEN-SHALOM[1], C. M. KEESHEN[1], J.-Y. AN[2], K. BERRIOS[3], S. SANDERS[2], K. J. BENDER[1];
[1]Neurol., [2]Psychiatry, UCSF, San Francisco, CA; [3]Chem., Univ. of Puerto Rico, Rio piedras, Puerto Rico

Abstract: Heterozygous mutations in *SCN2A,* identified by exome sequencing of 5,563 autism spectrum disorder (ASD) cases in the Simons Simplex Consortium (Iossifov et al., 2014) and Autism Sequencing Consortium (De Rubeis et al., 2014) demonstrate strong ASD association (Sanders et al., 2015). *SCN2A* encodes the alpha subunit of $Na_V1.2$, a voltage-gated sodium channel that is involved in action potential generation at the axon initial segment of glutamatergic neurons. Along with four *de novo* loss of function (LoF) mutations, *SCN2A* is the only gene in the genome with a clear excess of *de novo* missense mutations in ASD. The eight missense mutations, many of which are clustered near the ion selectivity pore, represent a 36-fold increase over expectation ($p = 1x10^{-10}$, binomial distribution). Whether these missense mutations alter the function of translated NaV1.2 channels remains unclear. Here, we used heterologous expression to examine electrophysiological properties of NaV1.2 channels mutated to contain ASD-associated LoF and missense mutations. Further, channel expression and membrane association were assessed by visualizing immunostained NaV1.2 channels using confocal and total internal reflectance (TIRF) microscopy. HEK293 cells were transfected with plasmids that allowed for expression of the wild type or mutated alpha subunit, GFP, and accessory beta subunits. Interestingly 5/8 ASD-associated missense mutations resulted in a complete loss of channel conductance, despite normal membrane association. Mutations in two other sites reduced peak current amplitude, and we are currently working to determine the biophysical basis for these changes. Based on these results, we are developing compartmental models that incorporate LoF and missense mutations to determine how ASD-associated changes in NaV1.2 function affect neuronal excitability. This work therefore identifies SCN2A as having the most mutations and strongest evidence of ASD association of any gene observed in the exome.

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Poster

680. Physiological Mechanisms in Autism and Autism Models

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Program#/Poster#: 680.02/E20

Topic: A.07. Developmental Disorders

Support: RGPIN341498

CCl117959

Title: The role of PAK signaling in the regulation of synaptic plasticity and social memory

Authors: ***C. LEUNG**[1,2], F. CAO[1,2], Z. P. JIA[1,2];
[1]Neurosciences and Mental Hlth., Hosp. For Sick Children, Toronto, ON, Canada; [2]Physiol., Univ. of Toronto, Toronto, ON, Canada

Abstract: Neurodevelopmental disorders including Autism spectrum disorders (ASD) and intellectual disability (ID) are characterized by social impairments that impact adaptive functioning. PAKs (p21-activated kinase) are a family of serine/ threonine protein kinases that are central regulators of actin cytoskeleton reorganization and neuronal morphology, and are involved in synaptic and behavioural plasticity. Recent genetic screening and post-mortem studies have implicated mutations in the PAK gene in ASD and ID, but whether and how PAKs are involved in social behavior remains unclear. To directly address this question, we have generated a novel transgenic mouse model using the tetracycline operator system (tet-off) where the expression of a dominant negative PAK mutation can be inducibly controlled. We find that the transgenic mice showed specific deficits in social memory but normal sociability and novelty recognition. These mice also exhibited impairments in synaptic transmission and plasticity. The deficits were fully rescued through the administration of a tetracycline analog, doxycycline. Together, these results suggest a critical role of PAK signaling in social behavior in mice. Supported by CIHR and NSERC.

Disclosures: **C. Leung:** None. **F. Cao:** None. **Z.P. Jia:** None.

Poster

680. Physiological Mechanisms in Autism and Autism Models

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 680.03/E21

Topic: A.07. Developmental Disorders

Support: Simons Foundation SFARI 368406

Title: Functional Assessment of ASD-associated variants in the PTEN gene

Authors: *K. POST, B. P. YOUNG, B. CALLAGHAN, M. BELMADANI, S. ROGIC, C. RANKIN, S. BAMJI, T. O'CONNOR, D. ALLAN, P. PAVLIDIS, C. LOEWEN, K. HAAS; Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Although progress has been made in identifying genes associated with autism spectrum disorder (ASD), the underlying mechanism of this disorder remains unknown. Recently, several exome-sequencing studies of thousands of patients and their families have been completed yielding hundreds of genes loosely linked to ASD, and a smaller subset repeatedly found to be mutated in ASD probands, indicating their likely association with this disorder. The research community is now faced with the daunting task of determining how multiple genes give rise to the disease. To address this problem, a two-stage strategy was used to capitalize on rigorous bioinformatics and high-throughput biological systems to screen for gene mutations most likely to provide strong phenotypes in a secondary slower throughput assay. The gene PTEN (phosphatase and tension homolog) was selected as the first gene to screen in these assays due to its rank as a high confidence candidate gene for ASD. PTEN and its variants, selected based on rigorous bioinformatics, were expressed in high-throughput assays in yeast. Using the synthetic gene array assay, genetic interaction profiles were analyzed to understand altered molecular function. This will inform how variants disrupt protein function and identify mutations producing the strongest phenotypes. The effect of a reduced set of variants on experience-driven growth, synaptogenesis, and neural encoding in vivo in awake, transparent *Xenopus laevis* tadpoles was analyzed. This powerful vertebrate system provides sensitive measures of precisely how ASD gene variants alter brain circuit development. This strategy provides insight into underlying molecular pathways mediating pathophysiology, neuronal and neural circuit development.

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Poster

680. Physiological Mechanisms in Autism and Autism Models

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Ministry of Education, Culture, Sports, Science and Technology in Japan Grants-in-Aid for Scientific Research 23111005

Title: Deficits in cerebellar synapse and circuit function in an autism mouse model for the human 15q11-13 duplication

Authors: *D. H. SIMMONS[1], C. PIOCHON[2], T. TAKUMI[3], C. HANSEL[4];
[1]Dept. of Neurobio., Univ. of Chicago Dept. of Neurobio., Chicago, IL; [2]Dept. of Physiol., Northwestern Univ., Chicago, IL; [3]RIKEN Brain Sci. Inst., Wako, Japan; [4]Dept. of Neurobio., Univ. of Chicago, Chicago, IL

Abstract: Autism Spectrum Disorder (ASD) is characterized by two hallmark symptoms: impaired social interaction and increased repetitive behaviors. While ASD is typically regarded as a social disorder, about 80% of patients also display motor learning deficits, suggesting involvement of the cerebellum in ASD pathology. We studied cerebellar physiology in the 15q11-13 copy number variation mouse model, which is the most frequent and penetrant genetic aberration seen in ASD. In these mice, genetic imprinting determines whether offspring will show pathology associated with the autistic-like phenotype. A mouse receiving the 15q11-13 duplication paternally (patDp/+), but not maternally (matDp/+), will show ASD-resembling behaviors (Nakatani et al., *Cell* 137, 2009). To investigate cerebellar abnormalities associated with this phenotype, including the known impairment of LTD-induction at parallel fiber (PF) – Purkinje cell synapses in patDp/+ mice (Piochon et al., *Nat. Commun*. 5, 2014), we studied

climbing fiber (CF) – Purkinje cell and PF – Purkinje cell synaptic transmission. patDp/+ mice showed abnormally large amplitude CF-evoked excitatory postsynaptic currents, which led us to look for altered synaptic density between CFs and Purkinje cells. Immunohistochemistry with VGluT2, a marker for CF terminals, indicated that patDp/+ mice displayed increased density of CF terminals on both large-caliber and fine Purkinje cell dendrites, the latter of which are ordinarily considered PF input territory. We next examined calcium transients in spines, fine dendrites, and large-caliber dendrites, evoked by paired stimulation of PF 100Hz with a single CF stimulation, a single CF stimulation, or PF 100Hz stimulation. Although the CF-evoked calcium transient was unaltered in patDp/+ and matDp/+ mice, our calcium imaging results revealed abnormally small amplitude calcium transients in response to paired stimulation and PF 100Hz stimulation in patDp/+ spines and fine branches. These data suggest that the underlying cause of the known LTD-induction impairment in patDp/+ mice may be due to weak, abnormally small calcium signaling at PF-Purkinje cell spines. It is possible that PFs, even with paired CF-stimulation, do not provide a sufficient calcium signal to induce LTD. The result of increased CF-Purkinje cell synapse density with reduced PF calcium transients raises the possibility that CF synapses are too abundant on Purkinje cell fine dendrites, and thereby invade territory typically reserved for PF-Purkinje cell synapses. Taken together, our findings highlight cerebellar physiological abnormalities that contribute to motor deficits in a mouse model of ASD.

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Poster

680. Physiological Mechanisms in Autism and Autism Models

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Program#/Poster#: 680.05/E23

Topic: A.07. Developmental Disorders

Support: NIH 5 F32 DC014887-02

Title: Altered connectivity of neonatal subplate and layer 4 neurons in primary auditory cortex in a mouse model of autism

Authors: *D. NAGODE[1], P. O. KANOLD[2];
[1]Biol., College Park, MD; [2]Biol., Univ. of Maryland Col. Park, College Park, MD

Abstract: Autism Specturm Disorder (ASD) is thought to be of neurodevelopmental origin, though the causes are unclear. Recent brain imaging studies have revealed significant abnormalities in the way that autistic individuals process speech and sound. Specifically,

temporal (including auditory) cortical areas exhibit different patterns of activation compared with typically developed individuals, suggesting that improper “wiring” in areas during development might contribute to problems in speech perception or interpretation. One potential cause for autism is in utero insults. For example prenatal exposure to the antiepileptic drug valproic acid (VPA), increases the incidence of ASD in humans and fetal VPA exposure also results in autistic phenotypes in rodents. The peak vulnerability for VPA in rodents is in the second gestational week thus the neural circuits affected are those present at this time. In the fetal cortex, the first functional circuits are formed by subplate neurons (SPNs). SPNs are crucially involved in key steps of cortical maturation, particularly processes in layer 4 (L4) such as the functional maturation of thalamocortical and intracortical wiring and in critical period plasticity. The vulnerability window for VPA to cause autistic symptoms coincides with the peak generation window of SPNs, thus SPN disruption after VPA might lead to later cortical dysfunction. We investigated in vitro the functional connectivity of SPNs and L4 neurons in neonatal and adult auditory thalamocortical slices using whole-cell patch clamp electrophysiology and laser scanning photostimulation (LSPS) of caged glutamate. VPA, at a dose of 500 mg/kg, resulted in altered excitatory and inhibitory connecions in subplate neurons and L4 neurons. Thus, prenatal exposure to VPA causes disruptions in early SPN circuits that are later reflected in permanent deficits in both excitatory and inhibitory connectivity in L4. Our results provide direct evidence that the earliest cortical auditory circuits are already disrupted in rodent models of autism, and suggest that dysfunction in transient SPN circuits in the developing brain might play a key role in the etiology of ASD.

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Poster

680. Physiological Mechanisms in Autism and Autism Models

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Topic: A.07. Developmental Disorders

Title: Cntnap2 -/- autism model mice display deficits in tonic and phasic inhibition in primary visual cortex

Authors: *M. BRIDI, S. PARK, S. HUANG;
Neurosci., Hussman Inst. For Autism, Baltimore, MD

Abstract: Investigations into the genetics of autism in human patients strongly suggest that changes in synaptic function are a common factor in autism pathophysiology. Evidence from studies in both humans and animal models suggest that this synaptic dysfunction leads to

excitatory/inhibitory (E/I) imbalance in the form of reduced inhibition and/or over-excitation, and that neuronal inhibition may be impacted by both pre- and post-synaptic changes. *Cntnap2* -/- mice are a well-established model of ASD. Numerous reports have found that these mice exhibit autism-like behaviors including reduced social interaction, hyperactivity, and repetitive/stereotyped behavior. In addition, *Cntnap2* -/- mice exhibit aberrant neuronal migration, reduced interneuron numbers, seizure activity, and alterations in synaptic spines. Here, we examined tonic and phasic inhibition in L2/3 pyramidal cells in primary visual cortex (V1) of *Cntnap2* -/- mice compared to wild-type (WT) controls, in two different age groups, 3-4 weeks and 6-8 weeks of age. We found that L2/3 pyramidal cells from 6-8 week old *Cntnap2* -/- mice exhibited significant smaller inhibitory tonic conductance. In 3-4 week old mice we found no significant effect of genotype on tonic inhibition. We also analyzed sIPSCs in both genotypes and age groups. While no differences were seen in sIPSC amplitude, frequency, or kinetics in 3-4 week old mice, we found an age-dependent effect of genotype in 6-8 week old animals, with lower sIPSC frequency in *Cntnap2* -/- mice. We did not observe an effect of age or genotype on resting potential or intrinsic excitability of L2/3 pyramidal cells in area V1. These data indicate that network-level GABAergic activity is disturbed in *Cntnap2* -/- autism model mice, which dovetails with previous reports of reduced interneuron numbers, altered network activity, and seizure susceptibility. Our findings suggest that reduced tonic inhibition could underlie autism-like behaviors, and future studies should investigate cellular/molecular mechanisms of reduced tonic inhibition as well as the effects of diminished inhibition on cortical function *in vivo*.

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Poster

680. Physiological Mechanisms in Autism and Autism Models

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Topic: A.07. Developmental Disorders

Support: Hussman Foundation Grant HIAS15007

Title: Comparative expression analysis of autism-associated cadherin superfamily members

Authors: *J. A. FREI, G. J. BLATT, Y.-C. LIN;
Neurosci., Hussman Inst. For Autism, Baltimore, MD

Abstract: Cell adhesion molecules (CAMs) play crucial roles in neural circuit formation. The cadherin superfamily is one of the largest families of CAMs containing more than one hundred molecules, including classical cadherins type I and II, protocadherins, and atypical cadherins.

Cadherins share a similar structure consisting of multiple EC-cadherin domains in the extracellular region to mediate Ca^{2+}-dependent adhesion. The cytosolic tail of cadherins interacts with catenins and small Rho GTPases to regulate actin dynamics. The type I classical cadherin N-cadherin is the most well-studied member to-date. N-cadherin functions throughout the development of the nervous system and is essential for synapse formation, spine morphogenesis, synaptic signaling and plasticity. In contrast to N-cadherin, little is known about the function of other cadherins. A genome wide association study performed by the Hussman Institute for Human Genomics identified the classical cadherin type II CDH8, CDH9 and CDH11; the protocadherin family member PCDH9; the atypical cadherin FAT1 and the α-catenin CTNNA3 (αT-catenin) as candidate risk genes. This suggests that cadherin signaling pathways could be disrupted and may display increased vulnerability in autism. Here, we investigate how cadherins across the different subfamilies, as well as components of the cadherin signaling pathway, play a role in brain development. The findings provide insights toward understanding the etiology of autism.

To investigate the importance of cadherin signaling in autism, we analyzed the spatial and temporal expression patterns of different autism-associated cadherin family members in different brain areas, as well as their subcellular localization in neurons. Temporal expression analysis in the developing mouse brain revealed increased cadherin protein expression from P0 to P7. For some of the cadherins analyzed, elevated expression persisted throughout stages P14 and P21. An increased expression from P7 to P21 is consistent with the proposed functions in dendritogenesis and synaptogenesis. Furthermore, cadherins from differing subfamilies showed distinct but partially overlapping expression patterns in various brain areas implicated in autism. Taken together, our results show that cadherins from different subfamilies could potentially converge into common signaling pathways to regulate neural circuit formation. Perturbation of these pathways might result in disrupted dendrite formation, spine morphogenesis and synapse function, cellular phenotypes that have been linked to autism.

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Poster

680. Physiological Mechanisms in Autism and Autism Models

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Topic: A.07. Developmental Disorders

Title: Rescues behavioral deficits in SHANK3 knock-out mice by pharmacological enhancement of mGlu5 receptors

Authors: *C. K. JONES[1], C. VICIDOMINI[2], L. PONZONI[3], D. LIM[4], A. TOZZI[5], D. REIM[6], A. A. GENAZZANI[7], P. CALABRESI[5], M. SALA[3], M. SCHMEISSER[6], C. W. LINDSLEY[1], T. M. BOECKERS[6], C. SALA[2], C. VERPELLI[2];
[1]Vanderbilt Univ. Med. Ctr., Nashville, TN; [2]CNR Neurosience Inst., Milano, Italy; [3]BIOMETRA Univ. of Milan, Milano, Italy; [4]Dept. of Pharmaceut. Sciences, Univ. degli Studi del Piemonte Orientale "Amedeo Avogadro", Novara, Italy; [5]Univ. of Perugia, Dept. of Exptl. Med., Perugia, Italy; [6]Inst. for Anat. and Cell Biology, Ulm Univ., Ulm, Germany; [7]Dept. of Pharmaceut. Sciences, Univ. degli Studi del Piemonte Orientale, Novara, Italy

Abstract: The Shank3 protein is a scaffold protein that is located in the postsynaptic density (PSD) of excitatory synapses and is crucial for synapse development and plasticity. *SHANK3* genetic haploinsufficiency is thought to be the major cause of neuropsychiatric symptoms in Phelan-McDermid syndrome (PMS). In this study, we investigated the molecular mechanisms associated with the ASD-like behaviors observed in *Shank3Δ11*$^{-/-}$ mice in which exon 11 has been deleted. Our results indicate that Shank3 is essential to mediating mGlu5 receptor signaling by recruiting Homer1b/c to the PSD, specifically in the striatum and cortex. Moreover, augmenting mGlu5 receptor activity by administering the mGlu5 PAMs ameliorated the functional and behavioral defects that were observed in *Shank3Δ11*$^{-/-}$ mice, suggesting that pharmaceutical treatments that increase mGlu5 activity may represent a new approach for treating patients that are affected by PMS and *SHANK3* mutations.

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Poster

680. Physiological Mechanisms in Autism and Autism Models

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Topic: A.07. Developmental Disorders

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Shiners Hospitals

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Title: The number of parvalbumin-expressing chandelier and basket cells are differentially decreased in medial prefrontal cortex in autism

Authors: *V. MARTINEZ-CERDENO;
Pathology, UC Davis, Sacramento, CA

Abstract: Cortical interneurons have been linked with the altered balance of excitation / inhibition in the cerebral cortex that is present in autism. However, a specific subtype of interneuron had not been correlated with autism until we discovered that the number of parvalbumin (PV) expressing interneurons was decreased in the prefrontal cortex in autism. There are two PV+ interneuron subtypes: Basket (Bsk) cells and Chandellier (Ch) cells. Bsk cells innervate the soma and proximal dendrites of pyramidal neurons, while Ch cells innervate the initial segment of pyramidal neuron axon. Both Bsk and Ch cells are fast-spiking neurons that innervate a large number of pyramidal neurons, and therefore even a small decrease in Ch or Bsk cell number could critically impair pyramidal neuron output and regional cortical function. These cells account for only 1% of total neurons in the cerebral cortex, with Bsk cells more numerous than Ch cells. A specific marker that differentiates Bsk from Ch cells has not been devised. However, we have designed a method that allows us to discern between these two cell types. Our method is based on the differential expression of *Vicia villosa* lectin (VVA) by Bsk and Ch cells. VVA lectin is present in the perineuronal net surrounding Bsk cells. While the vast majority of Bsk cells can be labeled using VVA, Ch cells do not express VVA. We used PV and VVA double labeling, and based on exclusion distinguished Ch cells from Bsk cells in cortical slices of prefrontal cortex in autism and control groups. We then quantified the number of each cell type (PV+/VVA+; PV+AVV-) present in each Brodmann area in our tissue samples and compared data between autism and control groups. We found that PV+ Ch cells (VVA-) are decreased in prefrontal BA46, BA47, and BA9. PV+ BsK cells may be also decreased but to a lesser degree in these areas. The changes in PV+ Ch and PV+ Bsk cells reported here may reflect altered information processing within the PFC and could contribute to the cognitive impairments seen in autism.

Disclosures: V. Martinez-Cerdeno: None.

Poster

680. Physiological Mechanisms in Autism and Autism Models

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Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 680.10/E28

Topic: A.07. Developmental Disorders

Title: Human tuberous sclerosis (TSC) 2 (-/-) genome-edited neural stem cells and neurones exhibit inhibited autophagy which is restored following mTORC1 inhibition

Authors: *L. STEWARD, N. ANASTASI, A. MARCUZ, N. BE, M. GURIDI ORMAZABAL; Roche Innovation Ctr. Basel, Neurosci. Discov, Basel, Switzerland

Abstract: mTORC1 regulates autophagy and in some neurodevelopmental disorders, such as tuberous sclerosis (TSC), mTORC1 hyperactivity leads to reduced autophagy which likely contributes to disease pathology. This is supported by evidence of impaired autophagy in tuberous sclerosis (TSC) patient cortical tuber tissue, as well as from rodent TSC knockdown studies (e.g. Di Nardo et al., 2014; McMahon et al., 2012) where p62 (a marker of protein build up and impaired autophagy) are increased when compared to control tissues. Although the TSC patient genotype is heterozygous TSC2 (+/-), an additional somatic TSC mutation can result in a knockout. We generated human TSC2 genome-edited neural stem cells (TSC2 NSCs) (+/+) wild type, (-/-) knockout and (+/-) heterozygote cells. These cells have many of the characteristics of patient cortical tubers (Costa et al., 2016). In this study we examined p62 levels and the effect of rapalog treatment in the TSC2-NSCs and their derived neurones, using western blots (WB) and a novel immunocytochemistry (ICC) assay using high content image analysis (HCS; Operetta). Preliminary studies confirmed mTORC1 hyperactivity, as rapamycin sensitive phospho-S6 was increased in TSC2 (-/-) NSCs and 1, 2, 3 and 4 wk old neurones, whereas TSC (+/-) neurones were similar to WT. P62 was significantly increased in TSC2 (-/-) NSCs and neurones compared to WT, whereas TSC (+/-) NSCs and neurones were not altered in WB or ICC HCS studies. In the ICC studies, it was apparent that there was a high level of diffuse P62 in the cytoplasm of the TSC2 (-/-) NSCs and neurones, which was decreased after 24h or 1 wk of rapamycin or everolimus treatment. Bafilomycin decreased the diffuse P62 and increased punctate p62 form (likely indicative of translocation from cytoplasm to autophagosomes and accumulation because of inhibited lysosomal degradation). This punctate P62 was further increased when combined with everolimus or rapamycin, indicating a treatment related increased autophagic flux after mTORC1 inhibition. Overall the data indicated that the TSC2 (-/-) NSCs and neurones are a good model for the human TSC patient cortical tubers, in that they mimic many aspects of the disease phenotype and specifically in this study, show similar mTORC1 dependent markers of dysfunction observed in patient cortical tubers.

Disclosures: **L. Steward:** A. Employment/Salary (full or part-time): Roche. **N. Anastasi:** A. Employment/Salary (full or part-time): Roche. **A. Marcuz:** A. Employment/Salary (full or part-time): Roche. **N. Be:** A. Employment/Salary (full or part-time): Roche. **M. Guridi Ormazabal:** A. Employment/Salary (full or part-time): Roche.

Poster

680. Physiological Mechanisms in Autism and Autism Models

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Topic: A.07. Developmental Disorders

Support: MH100600

MH104491

Title: Contributions of Cadherin-8 to establishing corticostriatal networks

Authors: *R. E. MESIAS, L. G. FRIEDMAN, D. L. BENSON, G. W. HUNTLEY;
Icahn Sch. of Med. At Mount Sinai, New York, NY

Abstract: Motor and executive tasks are supported by the development of functional corticostriatal networks. Nevertheless, little is known about the molecular mechanisms that control the establishment and function of these connections. Recently, we demonstrated that Cadherin-8 (Cdh8), a type II classic cadherin, was highly expressed in prefrontal cortex and dorsal striatum during an early postnatal period when cortical axons are growing into the striatum. Furthermore, using *in situ* hybridization techniques, we showed that Cdh8 is enriched in striatally-projecting pyramidal neurons in the medial prefrontal cortex and in striatal spiny projection neurons (SPNs). Knockdown of Cdh8 by RNA silencing in cultured cortical neurons or in SPNs *in vivo* significantly alters dendritic arborization and dendrite self-avoidance. Additionally, the temporal and spatial distribution patterns of expression strongly point toward a role for Cdh8 in development of corticostriatal synapses. We generated a conditional mutant mouse in order to selectively delete *Cdh8* from particular cell types. The effects of postsynaptic *Cdh8* deletion on synaptic transmission and plasticity were examined in SPNs using whole-cell voltage clamp recordings from wild-type and AAV-cre injected $Cdh8^{fl/fl}$ mice. These data reveal multifaceted roles of Cdh8 in regulating development of corticostriatal circuits. Elucidating the role of Cdh8 in circuit formation is essential as microdeletions in chromosome 16 have implicated *CDH8* in autism and learning disabilities.

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Poster

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Topic: A.07. Developmental Disorders

Support: Hussman Foundation - HIAS-15001

Title: The basal ganglia has altered inhibitory receptor expression in autism: implications for cortico-striatal dysfunction

Authors: *K. SUBRAMANIAN, C. BRANDENBURG, G. J. BLATT;
Neurosci., Hussman Inst. for Autism, Baltimore, MD

Abstract: The basal ganglia (BG) is a collection of highly interconnected sub-cortical nuclei that contain mainly inhibitory GABAergic medium spiny neurons (MSN's). The BG is connected to the cerebral cortex via the thalamus and the cerebellar cortex via the subthalamic nucleus (STN). Specific regions of the BG are implicated in stereotypy, cognitive behavior, motor, speech and language disorders. Thus, the BG is an ideal region of interest to examine the neurochemical basis of repetitive, stereotyped behavior and social communication difficulties observed in autism. This study examined changes to GABAA receptor expression in the BG of post-mortem brain tissue. Specifically, the dorsal striatum consisting of the caudate and putamen, and the ventral striatum including the core and shell territories of the nucleus accumbens (NAcc) and the subthalamic nucleus (STN) were quantified. Sampled regions in the dorsal striatum included projections from the dorsal anterior cingulate cortex (dACC), dorsolateral pre-frontal cortex (dlPFC), pre-motor, motor cortical areas and STN. Sampled regions in the ventral striatum included projections from ventromedial pre-frontal cortex vmPFC and orbitofrontal cortex (OFC) to the NAcc, areas that play a critical role in reward related and stereotypic behaviors. An autoradiographic assay with the ligand [^{3}H]-flunitrazepam was used to label benzodiazepine binding sites on GABAA receptors. There was a significant increase ($p<0.0001$) in GABAA receptor binding, measured in fmol/mg of protein, expressed as (mean ± sem) in age-matched control vs autism cases in dorsal striatum (57.63 ± 1.67, n=17 vs 92.80 ± 2.27, n=19), ventral striatum (98.98 ± 5.10, n=8 vs 161.21± 4.58, n=11) and a significant decrease ($p<0.0001$) in GABAA receptor binding in STN (12.56 ± 0.66, n=9 vs 7.48 ± 0.41, n=8). This initial GABAA receptor binding study suggests that there are significant increases in the expression of inhibitory receptors in the GABAergic regions of BG in autism. Conversely, a significant decrease of GABAA receptor binding was found in the glutamatergic STN region of BG. Taken together, this altered receptor expression pattern indicates a region-specific excitatory-inhibitory imbalance (E/I) in the BG in the autism cohort suggesting a dysregulation of key intrinsic BG circuits. It is unknown whether these changes are due to primary alterations in development or

compensatory due to the E/I imbalance. Further studies are underway to examine specific regions within the BG to better elucidate the extent of changes in cortical - BG connectivity and functions.

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Poster

680. Physiological Mechanisms in Autism and Autism Models

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Topic: A.07. Developmental Disorders

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Title: Loss of KIDLIA/KIAA2022 causes alterations in synaptic function, gene transcription and autistic behaviors in a novel autism mouse model

Authors: *J. P. GILBERT, H.-Y. MAN;
Biol., Boston Univ., Boston, MA

Abstract: Autism spectrum disorders (ASD) are characterized by impaired social activity, diminished communication and repetitive behavior. We have previously shown that loss of the ASD gene, KIDLIA/KIAA2022, impaired dendritic and axonal outgrowth *via* a disruption of N-cadherin/δ-catenin signaling. To further investigate the role of KIDLIA in neural development, we found that loss of KIDLIA expression resulted in a dramatic decrease in synapse formation and synaptic transmission, accompanied with a shift in the excitation/inhibition balance. RNA sequencing demonstrated that knockdown of KIDLIA altered the transcription of a large number of genes including those involved in synaptogenesis, synaptic plasticity and excitability. To further investigate the role of KIDLIA *in vivo*, we generated and characterized the first KIDLIA knockout mouse. The knockout mice showed significant impairments in social interactions, increased repetitive behaviors and deficits in learning and memory. The mice also demonstrated seizure activity and elevated anxiety. Therefore this new KIDLIA knockout mouse will be a valuable new model for autism research.

Disclosures: J.P. Gilbert: None. **H. Man:** None.

Poster

680. Physiological Mechanisms in Autism and Autism Models

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Title: Verification of abnormality of postnatal synapse formation/pruning in a primate model of ASD

Authors: *T. SASAKI[1,2], T. SANAGI[1], K. NAKAGAKI[1], T. MANABE[1], N. ICHINOHE[1]; [1]Dept. of Ultrastructural Research, Natl. Inst. of Neurosci., Natl. Ctr. of Neurol. and Psychiatry, Kodaira, Japan; [2]Lab. for Mol. Analysis of Higher Brain Function, Brain Sci. Institute, RIKEN, Wako, Japan

Abstract: The majority of excitatory synapses in the mammalian cerebral cortex occur at small protrusions, or spines, on the dendrites. In primates, the number of dendritic spines rapidly increases after birth, reaches a peak at around the early infancy or mid-childhood period, and then decreases towards the adult level. This characteristic “overshoot-type” profile is assumed to result from pruning of existing spines in excess of the generation of new spines in the later periods. Abnormalities of the processes of spinogenesis and pruning are implicated in several psychiatric disorders, such as autism spectrum disorder (ASD), schizophrenia, and Rett’s syndrome. In ASD, excess spine formation or incomplete pruning may occur, which leads to increased spine numbers.

We investigated the normal processes of spine formation/pruning in the cerebral cortex of the common marmoset (*Callithrix jacchus*) as a primate model. Our previous studies showed similarities and differences of developmental profiles of basal dendrites and spines of layer-III pyramidal cells among the six cortical areas (V1, TE, area 12, area 9, area 14r, area 24), and reported that all cortical areas showed overshoot-type spine formation with peaked at 2-3 month old (Oga *et al*., 2013, Sasaki *et al*., 2015).

We have developed ASD model of the marmoset by expose to valproic acid (VPA) during gestation period. The VPA marmoset has a deficit in communication and perseveration, which

are core features of ASD (Yasue *et al*., 2015). In this study, we measured the thickness of cortices, the density of cortical neurons, microglia, and the analyzed the processes of spine formation/pruning in the six cortical areas of the model animals. On 2, 3, and 6 month old, the thickness of gray matter was found to be increased in the VPA-exposed animals compared to that in unexposed (UE) animals. The density of cortical neurons and microglia was lower in the model animals than that in the UE animals. Layer-III pyramidal cells in the areas were intracellularly injected with Lucifer Yellow in lightly fixed slices, and reacted for Diaminobenzidine. Basal dendrites of more than 150 cells were reconstructed, and their morphological features were analyzed. In the prefrontal cortical areas, we found that insufficient spine pruning of the ASD model compared with UE animal. Search for the molecular mechanisms that underlie the developmental changes will provide further clues for an understanding of pathogenesis of ASD.

Disclosures: **T. Sasaki:** None. **T. Sanagi:** None. **K. Nakagaki:** None. **T. Manabe:** None. **N. Ichinohe:** None.

Poster

680. Physiological Mechanisms in Autism and Autism Models

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 680.15/E33

Topic: A.07. Developmental Disorders

Title: The autism associated gene cntnap3 is a critical cell-adhesion molecule in neurite and synapse development

Authors: *D. TONG;
Inst. of Neurosci., Shanghai City, China

Abstract: Autism spectrum disorders (ASD) is one kind of heterogeneous neurodevelopmental syndrome. These years, more and more genes have been reported to be predisposed to ASD, and many of them transcribe cell-adhesion molecules, such as *contactin associated protein-like* (*CNTNAP*), a superfamily of *neurexin* (*NRXN*). Here, we constructed a mouse model which *CNTNAP3* gene was knockout. The *CNTNAP3* KO mice showed deficits in social interaction and displayed abnormal learning and memory. The *in vitro* experiments revealed that *CNTNAP3* was required for the establishment of neural networks. RNAi-mediated knockdown of *CNTNAP3* induced the decrease of axon and dendrite length. We also constructed *CNTNAP3* KO rats which showed decreased spine density in CA1 pyramidal neurons and increased GABAergic interneuron density specifically in CA1 zone. Otherwise, the *CNTNAP3* KO rats also manifested the deficits in E/I balance. Our data suggest that *CNTNAP3* is a critical gene for neurite and

synapse development and provide another important model for investigating the mechanisms and therapeutic strategies of ASD.

Disclosures: D. Tong: None.

Poster

680. Physiological Mechanisms in Autism and Autism Models

Location: Halls B-H

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Program#/Poster#: 680.16/E34

Topic: A.07. Developmental Disorders

Support: Hussman Foundation Grant HIAS15003

Title: Autism-associated mutations in cep290 disrupt cell proliferation process

Authors: *M. B. KILANDER, Y.-C. LIN;
Neurosci., Hussman Inst. For Autism, Baltimore, MD

Abstract: Autism is classified as a neurodevelopmental condition, but the defined spatiotemporal molecular mechanisms contributing to the altered neurophysiology observed in individuals with autism are still largely unknown. In large-scale genetic analysis for autism risk genes, CEP290, a centrosomal protein has been identified as one of the candidate genes. Mutations in the *CEP290* gene are common in ciliopathies; severe multi-organ disorders caused by primary cilia dysfunction. The primary cilium is a microtubule rich cell protrusion crucial for normal cell migration, polarity and division. Moreover, the primary cilium serves as the confined compartment for selective cell signaling and for cell-environment communication. Sonic Hedgehog (Shh) signaling, a biological pathway necessary for proper tissue development and maintenance, is preferentially localized to the primary cilium and is essential for proliferation of granule cell progenitors (GCP) during cerebellar development. Interestingly, defects in cerebellar development occur frequently in CEP290 associated ciliopathies. However, how mutations of CEP290 may contribute to autism phenotypes is still unknown. Here, we test the hypothesis that autism-associated mutations in CEP290 alter the primary cilium in cerebellar GCPs and affect their proliferation during development.
First, the temporal and spatial expression of CEP290 in mouse brains was determined. CEP290 protein levels increase during normal postnatal development and it is highly expressed in the cerebellum compared to other brain regions. In Neuro-2a cells and cultured cerebellar GCPs, CEP290 is observed at the primary cilium and in the cytosol, displaying a dynamic, cell cycle-dependent, re-distribution pattern. Overexpression of CEP290 increased the rate of cell proliferation while CEP290 knockdown resulted in delayed cell population growth. Additionally,

alteration of the CEP290 level reduced neurite complexity in Neuro-2a cells. Furthermore, following the introduction of autism-associated CEP290 mutant constructs, the cell proliferation process was perturbed resulting in slower rate of cell division possibly due to disruption of primary cilium function ultimately leading to reduced Shh-mediated cell signaling events. In summary, CEP290 regulates cell proliferation, with additional effects on neurite complexity. Autism-associated mutations of CEP290 reduce cell proliferation. Our present investigation into the cellular functions of CEP290 offers novel insight into the role of the primary cilium in neurodevelopmental conditions including autism.

Disclosures: M.B. Kilander: None. **Y. Lin:** None.

Poster

680. Physiological Mechanisms in Autism and Autism Models

Location: Halls B-H

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Topic: A.07. Developmental Disorders

Support: ANR-11-LABX-0042

ANR-11-IDEX-0007

Title: Oxytocin fails to enhance serotoninergic neurotransmission in autistic patients

Authors: *A. Q. LEFEVRE[1,2], R. MOTTOLESE[2], J. REDOUTÉ[3], N. COSTES[3], D. LE BARS[3], M.-M. GEOFFRAY[2], M. LEBOYER[4], A. SIRIGU[2];
[1]CNC, CNRS, Bron Cedex, France; [2]Inst. des Sci. Cognitives Marc Jeannerod, Bron, France; [3]CERMEP, Bron, France; [4]INSERM, U841 Hôpital Chenevier-Mondor, Créteil, France

Abstract: A lot of efforts are currently made to evaluate the efficiency of oxytocin, a neuropeptide that can improve social behavior in Autism Spectrum Disorders (ASD). However, behavioral effects are often small and it is unclear how this molecule acts in the patients' brain. Some of oxytocin actions on social behavior are known to happen through an interaction with serotonin neurotransmission in animals and healthy humans. Critically, both oxytocinergic and serotonergic systems are suspected to be altered in ASD patients. We investigated oxytocin-serotonin interaction in 18 high functioning autistic male patients and 24 healthy controls (HC) using PET scan. With the radiotracer [18F]MPPF, a selective serotonin 1A receptor (5-HT_{1A}R) antagonist, we compared [18F]MPPF binding potential in both groups at baseline and after a spray containing placebo or oxytocin (24IU). Blood samples were also collected to evaluate the impact of oxytocin on peripheral serotonin.

Results showed no differences of [18F]MPPF binding potential at basal levels between patients and HC. In contrast, while oxytocin increased [18F]MPPF binding potential and serotonin peripheral levels in HC, we found no oxytocin effects in ASD patients. Our findings suggest that 5-HT_{1A}R density is normal in autistic patients, but their functioning might be disrupted, and thus oxytocin-serotonin interaction cannot occur in this pathology. This is in line with results from recent clinical trials showing moderate effects of oxytocin and suggests that therapeutic impact of this molecule alone may be limited. Our results thus open ways to investigate combined oxytocin-serotonin treatments.

Disclosures: A.Q. Lefevre: None. **R. Mottolese:** None. **J. Redouté:** None. **N. Costes:** None. **D. Le Bars:** None. **M. Geoffray:** None. **M. Leboyer:** None. **A. Sirigu:** None.

Poster

680. Physiological Mechanisms in Autism and Autism Models

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Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 680.18/E36

Topic: A.07. Developmental Disorders

Support: NIH 5T32NS061764-07

Title: Neuronal overexpression of β-catenin results in severe intellectual disabilities and autism

Authors: *J. ALEXANDER, S.-X. JIN, L. FEIG, M. H. JACOB;
Neurosci., Tufts Univ., Boston, MA

Abstract: Intellectual disabilities (ID) and autism spectrum disorders (ASD) are prevalent in the general population. Treatments are lacking because these disorders are molecularly ill-defined. Recent advances suggest that the hundreds of human ASD and ID-linked genes converge on a few key biological processes in neurons that predispose to disease. Our studies focus on the role of the β-catenin (β-cat)/ canonical Wnt signal transduction pathway in cognition and social behaviors. β-cat functions in both cadherin synaptic adhesion complexes and canonical Wnt target gene expression; these pathways modulate synaptic density, maturation, and plasticity, and are essential for proper brain function. Several human ID- and ASD-linked genes are predicted to cause either loss- or gain-of-function of the β-cat/ canonical Wnt pathway. However, direct tests that β-cat malfunction can cause these disorders, and knowledge of the underlying pathophysiological changes, are largely lacking. Our recent studies show that targeted deletion in mouse neurons of adenomatous polyposis coli protein (APC), a major negative regulator of β-cat levels, leads to elevated β-cat, altered synaptic density and function, cognitive deficits and autism-like behaviors (reduced social interest, increased repetitive behaviors). We propose that

among the downstream effects of APC loss, excessive β-cat is the major cause of these phenotypes. To test this, we have created a mouse that has targeted deletion of the degradation domain of β-cat, resulting in a stabilized protein product even in the presence of APC. Our new β-cat overexpressor mouse shows elevated β-cat levels in the brain, comparable to that of APC cKOs, and similar autism-like behaviors. However, β-cat overexpressor mice show more severe cognitive deficits, drastically reduced synaptic plasticity, severe reductions in AMPAR and NMDAR receptor levels, and an unanticipated upregulation of APC. We are identifying several, novel molecular and plasticity changes caused by aberrant β-cat levels in neurons. Our findings suggest new molecular etiologies that can cause cognitive deficits of different severities and autistic disabilities.

Disclosures: J. Alexander: None. **S. Jin:** None. **L. Feig:** None. **M.H. Jacob:** None.

Poster

680. Physiological Mechanisms in Autism and Autism Models

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 680.19/E37

Topic: A.07. Developmental Disorders

Support: Hussman Foundation

Title: Decreased parvalbumin mRNA levels in Purkinje cells in cerebellar Crus II in autism: implications for altered excitatory/inhibitory balance in fronto-cerebellar circuitry

Authors: *G. J. BLATT[1], S. REPRAKASH[2], K. ZHANG[2], J.-J. SOGHOMONIAN[2]; [1]Hussman Inst. For Autism, Baltimore, MD; [2]Anat. and Neurobio., Boston Univ. Sch. of Med., Boston, MA

Abstract: The most consistent neuropathological finding in postmortem brains from individuals with autism is decreased numbers of GABAergic cerebellar Purkinje cells (PCs), most pronounced in the posterior lobe lateral hemispheric region. The Crus II area, located immediately inferior to the horizontal fissure, has strong connections with prefrontal cortical areas that participate in high order cognitive tasks. A previous study from our laboratory reported that the remaining PCs in the Crus II region had significantly decreased GAD67 mRNA levels, the key synthesizing enzyme for GABA (Yip et al., Acta Neuropathol., 2007, 113(5):559-68). Most PCs also express the calcium-binding protein parvalbumin (PV). Physiologically, PV-containing neurons are fast spiking and exert powerful inhibitory effects on the soma and initial axon segment of their target neurons. For Crus II PCs, the main target neurons are those in the dentate nuclei that contain both excitatory and possibly inhibitory projection neurons, as well as

inhibitory interneurons. The current study further examined PV mRNA levels in Crus II area of control and autistic brains using radioisotopic in situ hybridization histochemistry and quantification of labeling at the single cell level. Results indicate that PV mRNA levels are significantly decreased in autism compared to control cases (mean (±SEM) and was respectively 114.7±14.48 vs. 191.6±32.13; n=10). mRNA values were not correlated with age at death or post-mortem interval in either group. In addition, there were no significant differences in PV mRNA levels between autism cases with reported seizures or not (respectively 148.7±49.51 vs. 127.5±27.62; n=5). A decrease in the number of PV neurons has been recently documented in prefrontal cortex of autistic brains (Hashemi et al., Cereb. Cortex. 2016). Results in our study provide new evidence that altered PV expression could contribute to the pathophysiology of the cerebellum and possibly to the pathophysiology of cerebellar-prefrontal circuits in autism. A role of PV in autism is consistent with a recent animal study showing that brain-wide metabolic dysfunction of PV neurons leads to impaired sensory gating and social ability in the mouse (Inan et al., Neurobiol Dis., 2016, 93:35-46). We are currently investigating the possibility that a deficit in PV expression affects other brain regions in autism.

Disclosures: **G.J. Blatt:** None. **S. Reprakash:** None. **K. Zhang:** None. **J. Soghomonian:** None.

Poster

680. Physiological Mechanisms in Autism and Autism Models

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 680.20/E38

Topic: A.07. Developmental Disorders

Title: Changes to microglial but not oligodendrocyte density in the posterior parietal cortex of autistic individuals

Authors: *M. S. MANIERKA, J. J. HUTSLER;
Integrative Program in Neurosci., Univ. of Nevada Reno, Reno, NV

Abstract: A variety of cortical changes associated with autism spectrum disorders (ASD) indicate disruptions to cellular patterning during neurodevelopment, including evidence of altered laminar structure, abnormal cortical minicolumns and possible deficits in synaptic pruning. Microglia play a variety of developmental roles, including; supporting neuronal health, synaptic growth, and synaptic pruning, in addition to acting as the primary immune defenders of the central nervous system. Given their importance in neurodevelopment and the prevalence of immune disturbances comorbid with ASD, it has been suggested that microglia may be critical to the etiology of this disorder. Previous research has found support for an increase in immune-

activated microglia in the prefrontal cortex and increased density of microglia in ASD grey matter (Morgan, JT, et al. 2010). However, the ionized calcium binding antigen that has been used as a microglia marker in previous research is heterogeneously expressed across microglial morphologies and is most strongly expressed in activated microglia. Staining methods relying on this marker may therefore have an inherent bias towards labeling of activated microglia. Thus, it is possible that previous reports of increased microglial density indicate increased conversion to an activated state rather than increased proliferation.
To explore this possibility, we compared cortical densities of microglia and oligodendrocytes in the supragranular layers (layers II and III) of posterior parietal cortex (Brodmann area 7) in four autistic males and five age- and sex-matched neurotypical controls. Tissue blocks were sectioned at 25µm across the cortical layers and stained with thionin to label Nissl bodies. In order to estimate cell population densities, counts were acquired from multiple tissue sections taken from each block using the optical dissector method (Stereologer, SRC Inc.). Microglial density was significantly increased within the ASD sample. Our findings support a possible general increase in microglial proliferation and density in the ASD cortex, which extends beyond microglial subtype and is not found in oligodendrocytes. Disturbances of microglial cell populations appear widespread in the ASD cortex, suggesting that altered microglial density may contribute to changes in synaptic and cellular patterning during cortical development.

Disclosures: **M.S. Manierka:** None. **J.J. Hutsler:** None.

Poster

680. Physiological Mechanisms in Autism and Autism Models

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Topic: A.07. Developmental Disorders

Support: Dutch Organisation for Medical Sciences (ZonMw)

NWO (ALW)

Neuro-Basic

ERC Advanced

Innovative Medicines Initiative Joint Undertaking under grant agreement n° 115300

Deutsche Forschungsgemeinschaft (DFG SFB1149)

Title: Dysfunctional cerebellar Purkinje cells contribute to autism like behaviours in Shank2-deficient mice

Authors: *S. PETER[1,2], M. M. TEN BRINKE[1], J. STEDEHOUDER[1], C. M. REINELT[3], B. WU[1], H. ZHOU[1], K. ZHOU[1], H. J. BOELE[1], S. A. KUSHNER[1], M. LEE[4], M. SCHMEISSER[3], T. M. BOECKERS[3], M. SCHONEWILLE[1], F. E. HOEBEEK[1], C. I. DE ZEEUW[1,2]; [1]Erasmus MC, Rotterdam, Netherlands; [2]Netherlands Inst. for Neurosci., Amsterdam, Netherlands; [3]Inst. für Anatomie und Zellbiologie, Ulm, Germany; [4]Yonsei Univ. Col. of Med., Seoul, Korea, Republic of

Abstract: Autism-spectrum-disorders (ASD) are neurodevelopmental disease entities primarily defined by deficits in social interaction and repetitive behaviour. In addition, individuals with autism often suffer from motor skill deficiencies, many of which manifest early in the disease. The aetiology of ASD is complex with reported pathophysiological alterations encompassing multiple brain regions, including the cerebellum. Cerebellum-related motor symptoms of ASD patients have been observed by impairments in eyeblink conditioning, eye movement abnormalities, general motor learning deficits as well as balance and postural difficulties. Loss-of-function mutations in the gene encoding the postsynaptic scaffolding protein SHANK2 are a highly penetrant cause of autism-spectrum-disorders (ASD) including cerebellum-related motor problems. Recent studies have implicated cerebellar pathology in the etiology of ASD. Using a KO mouse model we evaluated the possibility that cerebellar Purkinje cells represent a critical locus of ASD pathophysiology in SHANK2-related ASD. Absence of Shank2 impaired both Purkinje cell intrinsic plasticity and induction of long-term potentiation at the parallel fiber to Purkinje cell synapse. Moreover, inhibitory input onto Purkinje cells was significantly enhanced, most prominently in the posterior lobe where simple spike regularity was most affected. Using Purkinje cell-specific Shank2-knockout mice, we confirmed the in vivo alterations of simple spike regularity and the cerebellar-dependence of ASD-like behavioural phenotypes in motor learning and social interaction. These data highlight the importance of SHANK2 for Purkinje cell function, and support a model by which cerebellar pathology functions prominently in certain forms of ASD.

Disclosures: **S. Peter:** None. **M.M. Ten Brinke:** None. **J. Stedehouder:** None. **C.M. Reinelt:** None. **B. Wu:** None. **H. Zhou:** None. **K. Zhou:** None. **H.J. Boele:** None. **S.A. Kushner:** None. **M. Lee:** None. **M. Schmeisser:** None. **T.M. Boeckers:** None. **M. Schonewille:** None. **F.E. Hoebeek:** None. **C.I. De Zeeuw:** None.

Poster

680. Physiological Mechanisms in Autism and Autism Models

Location: Halls B-H

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Program#/Poster#: 680.22/F2

Topic: A.07. Developmental Disorders

Title: DNA methylation, hydroxymethylation and formylation in human frontal cortex of autistic and schizophrenic subjects

Authors: *R. C. DETH[1], M. S. TRIVEDI[2], Y. ZHANG[3], H. ABDOLMALEKY[4];
[1]Pharmaceut. Sci., Nova Southeastern Univ., Davie, FL; [2]Nova Southeastern Univ., Fort Lauderdale, FL; [3]Northeastern Univ., Boston, MA; [4]Boston Univ., Boston, MA

Abstract: Epigenetic regulation provides a molecular mechanism for long-lasting, activity-dependent changes in gene expression. In the brain, increased neuronal activity can translate into changes in DNA and histone methylation with epigenetic consequences, contributing to neuroplasticity and memory formation. The folate and vitamin B12-dependent enzyme methionine synthase (MS) controls methylation and is sensitive to redox status, linking aerobic metabolism to epigenetic regulation. In previous postmortem brain studies we showed that MS expression in frontal cortex decreases dramatically with age (500-fold), while levels of the MS cofactor methylB12 (methylcobalamin; MeCbl) decrease more than 10-fold across the lifespan. Furthermore, in autistic subjects MS expression and MeCbl levels were significantly lower than age-matched controls, in association with decreased levels of the methyl donor S-adenosylmethionine (SAM). MeCbl levels were also decreased in schizophrenia subjects. To determine whether these abnormal metabolic conditions are associated with epigenetic manifestations, we measured global levels of 5-methylcystosine (5mCyt), 5-hydroxymethylcytosine (5hmCyt) and 5-formylcytosine (5fCyt) in DNA from Brodmann areas 10/11 and 44/45 in frontal cortex. Levels of 5mCyt and 5hmCyt were higher in autistic subjects (4-11 yrs) vs. age-matched subjects (5-13 yrs), while levels of 5fCyt were lower. In schizophrenic subjects (36-49 yrs), levels of 5mCyt were lower than age-matched controls (36-50 yrs), while levels of 5hmCyt and 5fCyt were higher. A comparison of young vs. middle-age control subjects revealed significantly lower levels of 5mCyt and 5fCyt, while levels of 5hmCyt were higher in the older subjects. These findings confirm that abnormally decreased MS expression and MeCbl levels in frontal cortex of autistic and schizophrenic subjects are associated with differential 5mCyt, 5hmCyt and 5fCyt status, suggesting that epigenetic dysregulation may contribute to neurodevelopmental and neuropsychiatric disorders.

Disclosures: **R.C. Deth:** None. **M.S. Trivedi:** None. **Y. Zhang:** None. **H. Abdolmaleky:** None.

Poster

680. Physiological Mechanisms in Autism and Autism Models

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 680.23/F3

Topic: A.07. Developmental Disorders

Support: R01 AG044499

Title: Autism-associated Mint2 N723S mutation leads to disruption in neuronal development and synaptic function

Authors: *Y. LIN[1], C. KENNY[2], S. HENRY[2], U. BEFFERT[2], A. HO[2];
[1]Dept. of Pharmacol. and Exptl. Therapeut., [2]Biol., Boston Univ., Boston, MA

Abstract: Autism Spectrum Disorders (ASDs) comprise a heterogeneous group of neurodevelopmental disorders characterized by a complex genetic etiology and impairments in social skills, communication and repetitive behavior. Mutations in the human *APBA2*/*MINT2* gene that encodes for a neuronal adaptor protein have been genetically-linked to autism. Interestingly, the PDZ domain of Mint2 interacts directly with neurexin I, an ASD gene, as part of a multi-protein complex that acts as a facilitator of neurotransmitter release. Since Mints are multi-domain proteins and bind to several synaptic proteins, this raises the question whether Mint2 ASD mutants alter the binding and function to any of its interacting partners and affect Mint2 function. Here, we found the autism-linked Mint2 N723S mutation located within the second PDZ domain effectively binds neurexin I, but leads to reduce neurexin I protein stabilization and defective trafficking to the membrane in HEK293T cells. To determine the direct effects of Mint2 N723S mutant in synapse formation in primary neurons, we examined the heterozygous Mint2 N723S mutation phenotype in a Mint knockout background based on redundancy of Mint proteins. We found neurons expressing Mint2 N723S mutant blocked heterologous synapse formation induced by neuroligin-1 which correlated with decrease in miniature event frequency in excitatory synapses. In addition, we performed live imaging of GFP-tagged Mint2 wild type and Mint2 N723S mutant in primary neurons and observed the N723S mutant was less mobile which may alter the surface mobility of neurexins to the plasma membrane. Together, our results suggest that sequence N723S variant in Mint2 lead to dysfunction in synaptic formation, in part due to alterations in intracellular neurexin trafficking and altered synaptic function of Mint2 as potential mechanisms that contributes to ASD pathogenesis.

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Poster

680. Physiological Mechanisms in Autism and Autism Models

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Topic: A.07. Developmental Disorders

Support: Mitchell Center for Neurodegenerative Diseases

STARS Award

UT Start up funds

Title: Understanding mechanisms of susceptibility genes implicated in autism spectrum disorders

Authors: *K. R. BARBER[1], A. M. BUCKLEY[1], J. TANQUARY[1], M. WOODSON[2], M. B. SHERMAN[2], Y. P. WAIRKAR[1];
[1]Neurol., Univ. of Texas Med. Br. Dept. of Neurol., Galveston, TX; [2]Univ. of Texas Med. Br., Galveston, TX

Abstract: Autism spectrum disorders (ASD) are a heterogeneous group of neurodevelopmental disorders affecting one in every 68 children under the age of three. It is generally accepted that ASDs have a genetic component and many large-scale screens have identified several susceptibility genes involved in synapse development. However, the relationship between ASDs and synaptic dysfunction is poorly understood. In order to explore this relationship, we selected genes from the SFARI database that had not been strongly correlated with ASD and asked whether these genes might disrupt synaptic structure/function. For this, we utilized the *Drosophila* neuromuscular junction, which is a model of synapse development. Furthermore, Drosophila allows for powerful genetic manipulations, high-resolution single synapse studies and electrophysiological characterization that can be performed with relative ease. We found that some of these genes may disrupt axonal transport of synaptic proteins. These deficits in axonal transport could lead to improper development and malfunction of synapses, thereby affecting their normal neuronal function. These data will be further discussed during the conference.

Disclosures: K.R. Barber: None. **A.M. Buckley:** None. **J. Tanquary:** None. **M. Woodson:** None. **M.B. Sherman:** None. **Y.P. Wairkar:** None.

Poster

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Topic: A.07. Developmental Disorders

Support: NIH Grant MH071739

NIH Grant GM058234

Title: Altered responses of synaptic strength and intrinsic excitability to homeostatic challenges in Timothy Syndrome

Authors: *S. D. SUN[1,2], B. S. SUUTARI[1,2], B. LI[2], R. W. TSIEN[2];
[1]Ctr. for Neural Sci., New York Univ., New York, NY; [2]Neurosci. Inst., New York Univ. Med. Ctr., New York, NY

Abstract: Dysfunctional homeostasis has been suggested as a potential pathogenic mechanism underlying autism spectrum disorders (ASD). To explore this possibility, we have studied homeostatic regulation of synaptic transmission and intrinsic excitability in a mouse model (TS2-neo) of a monogenic form of ASD, Timothy Syndrome (TS). TS arises from mutations in the pore /forming subunit of the $Ca_V1.2$ calcium channel and recapitulates ASD behaviors.
We characterized differences between wild-type (WT) and the TS2-neo mouse model of TS with regard to homeostatic regulation of unitary neurotransmission and spike firing. We investigated changes in homeostatic synaptic plasticity by measuring miniature postsynaptic currents (mEPSCs) in dissociated primary cultures from cortices of heterozygous TS2-neo and WT siblings. Between 12-14 days *in vitro* (DIV), TS2-neo pyramidal neurons exhibited a lower basal frequency of events no difference in mEPSC amplitude compared to WT. After 24 h of action potential (AP) blockade by chronic tetrodotoxin (TTX) treatment, mEPSC amplitude increased in both TS2 and WT pyramidal neurons. Relative to WT, the TS2-neo pyramidal cells exhibited a significantly larger rightward shift in their amplitude distribution curve after TTX treatment, whereas mEPSC frequency was not different.
We also assessed homeostatic regulation of intrinsic excitability in pyramidal neurons, both in primary cortical cultures and *ex vivo* slices, by monitoring the alternative splicing of BK channels, a pivotal regulator for AP duration. As we describe elsewhere, chronic TTX treatment resulted in a prolongation in action potential attributed to the reduction in the inclusion of exon 29 (E29) in BK channel mRNA. Strikingly, the effect of inactivity was accentuated in TS2-neo neurons, just as for synaptic strength; in these neurons, E29 inclusion was significantly less than in WT E29 inclusion, both in cultures treated with TTX and in pyramidal neurons in contralateral visual cortex after 5 d monocular deprivation (MD) *in vivo*. We also observed electrophysiological differences (AP prolongation, increased excitability) in layer 2/3 pyramidal

neurons recorded *ex vivo* from MD animals.
These data reveal intriguing alterations in the homeostatic responses of TS2-neo pyramidal neurons, both *in vitro* and *in vivo.* The general pattern is that responses to homeostatic challenges are exaggerated in the mutant genetic context, both for synaptic events and neuronal properties. Our results encourage further investigation of homeostatic regulation in neuropsychiatric diseases and the hypothesis that dysfunctional homeostasis may contribute to ASD.

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Poster

680. Physiological Mechanisms in Autism and Autism Models

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Topic: A.07. Developmental Disorders

Support: NYU Finding a Cure for Epilepsy (FACES)

NIH grant NS074785

Title: Increased spontaneous activity and seizure susceptibility in a mouse model of Timothy Syndrome

Authors: *A. SALAH[1], O. DEVINSKY[2], D. M. TALOS[3], R. W. TSIEN[1];
[1]NYU Neurosci. Inst., [2]Comprehensive Epilepsy Ctr., New York Univ. Sch. Med., New York, NY; [3]Perelman Sch. of Medicine-Neurology, Univ. of Pennsylvania, Philadelphia, PA

Abstract: Timothy syndrome (TS) is a rare multisystem genetic disorder caused by mutations in the L-type calcium channel Cav1.2 and manifesting with cardiac arrhythmia, autism and epilepsy. We focused on an established TS mouse model (TS2-neo mice; Bader et al. 2011) that recapitulates critical autistic features of the human disease. These mice develop an autism phenotype prior to 3 months of age, but the nature and time course of abnormal electrical brain activity is not known. Accordingly, we investigated their spontaneous activity and seizure susceptibility under Kainic acid (KA) by performing long-term (6 h to 2 weeks) video-electroencephalography (V-EEG).
Methods: 3-6 month old mice under Isoflurane anesthesia were implanted with 6 epidural electrodes. In search of spontaneous activity, each animal was subjected to recordings for 60 h at 3 different points (20 h per session). For seizure susceptibility, animals were recorded for 2 h at baseline before KA administration (25mg/kg; i.p); after the injection mice were recorded for an additional 4 h to determine seizure latency (n=6 for each phenotype). Behavioral scoring for

seizure intensity after KA injections was performed with the Schauwecker and Steward Scale (1997). Seizure latency was determined along with the time spent to reach the first stage stage (S1) and the first tonic-clonic seizure (S4).
Results: Spontaneous activity was increased in the TS mice compared to controls as seen in the mean number of single spikes ($p<0.0005$), repetitive spikes ($p<0.02$), and runs of spikes ($p<0.0005$) over a 30 min period. Over 90 min, highly significant differences were found for all three conditions ($p<0.0001$) ($n=6$ for each phenotype). After KA injections, seizure susceptibility was greater in autistic mice compared to controls ($p<0.012$). Also, TS mice required less time to reach the first state (S1) ($p<0.0004$) or the first tonic-clonic seizure (S4) ($p<0.015$).
Conclusions: Our results support the relevance of the TS mouse for studying the mechanistic link between epilepsy and autism, and its possible usefulness as a model system for the testing of new therapeutics.

Disclosures: **A. Salah:** None. **O. Devinsky:** None. **D.M. Talos:** None. **R.W. Tsien:** None.

Poster

681. Animal Models of Environmental Effects on Neurodevelopment

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Topic: A.07. Developmental Disorders

Support: NIH Grant ES025585

NIH Grant MH018399

Title: Maternal immune activation leads to impairments in behavioral flexibility, repetitive grooming, and social approach deficits

Authors: ***D. A. AMODEO**, S. B. POWELL;
Univ. of California San Diego, San Diego, CA

Abstract: Epidemiological studies suggest that the risk of developing autism spectrum disorder (ASD) is increased by prenatal exposure to viral or bacterial infection during pregnancy. Individuals diagnosed with ASD have impairments in behavioral flexibility as evidenced by a probabilistic reversal learning deficit. Here we investigated how maternal immune activation (MIA) may impact behavioral inflexibility in mice in a spatial discrimination task using an 80/20 probabilistic reinforcement procedure. We hypothesized that MIA would lead to impaired probabilistic reversal learning, social approach deficits, and repetitive grooming which is associated with lower order repetitive behaviors expressed in ASD. MIA was initiated by

injecting pregnant C57BL/6J dams with 20 mg/kg polyriboinosinic-polyribocytidilic acid (poly I:C) in saline on gestational day 12.5. Control females were injected with saline. Both male and female pups born to poly I:C- or vehicle-treated dams were subsequently tested for ASD-relevant behavioral phenotypes, including social approach, repetitive grooming, and probabilistic reversal learning. Male and female offspring of MIA or control dams demonstrated comparable learning for the initial probabilistic discrimination. MIA led to impaired probabilistic reversal learning compared to controls in male mice but not female mice. Similarly, male MIA mice exhibited decreased social approach and elevated repetitive grooming compared to saline-treated control mice, while MIA females did not exhibit these phenotypes. These findings suggest that MIA during late gestation can lead to impairments in behavioral flexibility in male mice similar to that exhibited in ASD individuals. These findings provide a model for examining novel treatments for disorders such as autism that may be triggered by prenatal immune challenges.

Disclosures: D.A. Amodeo: None. **S.B. Powell:** None.

Poster

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Topic: A.07. Developmental Disorders

Support: This study is part funded by the MRC CIC scheme and by Roche

Title: Early neurodevelopmental consequences of maternal immune activation in male and female Wistar rats.

Authors: *J. NEILL[1], M. EDYE, MP13 9PT[2], K. MURRAY[3], M. HARTE[2], J. DENNISON[2], I. KNUESEL[4], E. PRINSSEN[4];
[1]Manchester Pharm. school, Univ. of Manchester, Oxford road, United Kingdom; [2]Univ. of Manchester, Manchester, United Kingdom; [3]Departments of Neurol. and Neurosci., Yale Univ., Newhaven, CT; [4]Roche Innovation Ctr. Basel, Basel, Switzerland

Abstract: Maternal immune activation (mIA) through administration of the viral-mimetic polyriboinosinic-polyribocytidylic acid (poly-I:C) is a key model for neurodevelopmental disorders such as schizophrenia (see Knuesel et al. 2014 for review). Gestational and early postnatal day neurodevelopmental changes in the offspring of poly I:C treated mothers have yet to be fully explored in rats. Our study aimed to establish an mIA model in rats and investigate early neurobiological markers in male and female offspring from poly I:C-treated rat dams. MIA was induced in pregnant Wistar rats with 10mg/kg poly I:C at gestational day (GD)15. Blood

samples were taken at 3h following poly I:C administration and changes in IL-6 expression measured in the plasma by ELISA. Offspring development was assessed in males and females at GD21 and postnatal day (PD)21. Brains were harvested for quantitative RT-PCR to measure changes in expression associated with neuronal development (myelin basic protein, MBP; myocyte enhancer factor-2, Mef2; major facilitator superfamily domain 2a, Mfsd2a; semaphorin 3a, Sema3a; shank3), glial cells (glial fibrillary acidic protein, GFAP; olfactomedin-like 3, Olfml3). Microglia in 30um hippocampal slices from male offspring were stained using Iba1. Values are means of multiple counting frames for 6 slices per brain. Counting areas were randomly placed over the hippocampus. Counting was performed by an experimenter blind to treatment. Number of total microglial cells counted classed as resting (A), intermediate (B) or ameboid (C) was calculated. Poly I:C at 10mg/kg on GD15 induced mIA without affecting litter numbers or maternal weight. However, at GD21, pups of poly I:C-treated dams displayed reduced weight, length, head circumference and placenta weight and this reduction in body weight was maintained in both genders at PD21. Elevated expression of Mef2 and Sema3a was observed in the frontal cortex of offspring of poly I:C-treated dams at both GD21 and PD21, whereas MBP expression was reduced at GD21 but elevated at PD21 and Mfsd2a expression reduced at GD21 only. We also observed significantly elevated levels of ameboid microglia in hippocampus on PD21. We have established a robust model of mIA in rats and used this to identify early neurodevelopmental changes. Poly I:C-induced mIA resulted in pup and placenta growth restriction alongside changes in markers suggesting delayed myelination and development of the blood-brain barrier but accelerated synaptic pruning and axonal guidance and activation of microglia. This work supports existing data in mice and further provides a model to study behavioural changes in rats alongside neuroimaging read-outs.

Disclosures: **J. Neill:** 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; current research funding from Takeda, GW Pharma, Roche, Autifony, Eisai, Boehringer Ingelheim. **M. Edye:** None. **K. Murray:** None. **M. harte:** None. **J. Dennison:** None. **I. Knuesel:** A. Employment/Salary (full or part-time): Irene Knuesel is a full-time employee of Roche. **E. Prinssen:** A. Employment/Salary (full or part-time): Eric Prinssen is a full-time employee and share holder of Roche.

Poster

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Topic: A.07. Developmental Disorders

Support: NIMH R21 MH105826

NARSAD Young Investigator Award

Title: Prenatal allergen exposure effects on microglia, dendritic spines in the prefrontal cortex, and cognitive flexibility

Authors: *K. M. LENZ[1], A. KRUG[2], A. JOSHI[2], A. GALAN[2];
[1]Psychology, Ohio State Univ. Dept. of Psychology, Columbus, OH; [2]The Ohio State Univ., Columbus, OH

Abstract: Perinatal inflammation increases risk for neurodevelopmental disorders, including autism, schizophrenia and ADHD, and both autism and ADHD are four times more common in males than females. Maternal allergic conditions during pregnancy increase the risk for ADHD and autism in children and allergic conditions are highly co-morbid with both ADHD and autism, but little is known about how allergic inflammation may alter brain development to increase risk for these neurodevelopmental disorders. In these experiments, we sought to determine whether prenatal allergic immune challenge shapes a.) innate immune cell number or activity in the developing brain, b.) locomotion, anxiety, cognitive flexibility, and social behavior and c.) dendritic spine density in the prefrontal cortex of male and female offspring. Adult female rats were sensitized to ovalbumin (OVA) via two injections with alum adjuvant, and then bred. On embryonic day 15 dams were challenged with intranasal OVA, which induced significant increases in maternal serum levels of IgE. On embryonic day 16 or the day of birth, brains of pups were assessed for innate immune cell numbers, both microglia (immunostained for the marker Iba1) and mast cells (stained with toluidine blue). Prenatal allergen exposure led to significant increases in microglial and mast cell number and activation in brains of both male and female offspring. A second cohort of OVA-exposed pups was grown to adulthood for behavioral testing and dendritic spine analysis. On the open field test, OVA offspring showed decreased anxiety and increased locomotor behavior. In juvenile social play testing, OVA challenged males showed decreases in sociality. Animals were then tested for cognitive flexibility and working memory on the attentional set shifting task, which is dependent on the prefrontal cortex. OVA-exposed offspring showed no deficits in simple discrimination, but showed significant deficits in reversal and extra-dimensional shifting, indicative of attentional deficits or cognitive inflexibility. Following behavioral testing, animals were sacrificed and their brains processed for Golgi-Cox staining and dendritic spine density assessed using Neurolucida software. Preliminary data shows that OVA exposure led to significant decreases in dendritic spine density in males, and significant increases in dendritic spine density in females. Together, these studies show that prenatal allergen exposure alters brain development and function and may be an underappreciated contributor to neurodevelopmental disorders.

Disclosures: **K.M. Lenz:** None. **A. Krug:** None. **A. Joshi:** None. **A. Galan:** None.

Poster

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Topic: A.07. Developmental Disorders

Support: Heart and Stroke Foundation of Canada G145756

Title: Placental group B Streptococcus infection: sex specific inflammatory response and autistic-like traits in male offspring

Authors: *M.-J. ALLARD[1], J. BERGERON[2], M. DESCOTEAUX[2], L. TREMBLAY[2], M. LEPAGE[2], L.-C. FORTIER[2], G. SÉBIRE[1,2];
[1]McGill Univ., Montreal, QC, Canada; [2]Univ. de Sherbrooke, Sherbrooke, QC, Canada

Abstract: Introduction: Group B *Streptococcus* (GBS) is infecting 15-30% of pregnant women, and is one of the major causes of chorioamnionitis, which is associated with preterm births and perinatal brain injuries leading to neurobehavioral impairments such as autism spectrum disorder (ASD). Our hypothesis is that end-gestational GBS infection of the dams impacts the placenta through an interleukin-1β (IL-1β)-driven inflammatory response, and that IL-1 driven chorioamnionitis leads to critical ASD network injuries. Using a rat model, our goal was to study the consequences of GBS infection on the placenta, and the subsequent neurodevelopmental effects in the offspring.
Methods: Lewis dams were inoculated intraperitoneally on gestational day (G) 19 with live serotype Ia GBS (10^8 CFU). Caesarian-sections were performed at multiple time points following the infection to collect placentas, and maternal and fetal blood samples. The maternofetal inflammatory response was studied by ELISA and immunohistochemistry. Behavioral tests were performed from postnatal day (P)7 to P40 to assess ASD-like behaviors. Brains were collected at P40 for histological studies. Magnetic resonance imaging and diffusion weighted imaging were performed on young adult rats.
Results: GBS placentas were infected, but did not result in pups' infection. Male offspring from GBS-exposed dams presented developmental impairments characterized by ASD-like behaviors with defective: communication, social interactions, and sensory integration. GBS-exposed dams displayed chorioamnionitis characterized by a higher infiltration of polymorphonuclear cells in male than female. Following GBS infection, increased titers of IL-1β were detected in maternal blood, male placentas, and male fetuses' blood, vs control tissues. At P40, GBS-exposed males showed a reduced thickness of the external capsule, of the frontal neocortex and of the corpus callosum, with a decreased mean fractional anisotropy in the anterior part of the corpus callosum. An increased thickness of the cingulum were observed in GBS-exposed males. None of these differences were observed in GBS-exposed females. Placental inflammation and forebrain

injuries will be further characterized by ongoing studies.
Discussion: Exposure to live GBS induces maternofetal immune activation resulting in neurodevelopmental abnormalities recapitulating those of human ASD, including sex dichotomy and behavioral phenotype. The results of the present study provide new evidence in favor of the role of a common and modifiable infectious/inflammatory environmental factor in human ASD pathophysiology.

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Poster

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Topic: A.07. Developmental Disorders

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Title: Neonatal infection with Borna Disease Virus causes widespread dendritic branching and spine neuropathology in brains of 3 week-old rats

Authors: *R. F. MERVIS[1], S. P. ZAJD[2], A. D. BACHSTETTER[3], K. DEMAYO[4], A. BARTON[4], E. ORELLANA[4], J. ESSA[4], A. SIDHOM[4], J. S. PADILLA[4], S. BAZZI[4], N. K. PATEL[5], N. NOBREGAS[4], W. I. LIPKIN[6], M. HORNIG[6];
[1]Neurostructural Res. Labs, Temple Terrace, FL; [2]Neuromorphology Study Group, Neurostructural Res. Labs, Tampa, FL; [3]Anat. & Neurobio., Univ. of Kentucky, Lexington, KY; [4]Col. of Arts & Sci., [5]The Honors Col., Univ. of South Florida, Tampa, FL; [6]Ctr. for Infection & Immunity, Columbia University, Mailman Sch. of Publ. Hlth., New York, NY

Abstract: There is much concern surrounding the neuropathological sequelae of gestational Zika virus infection in man. Here, we examined the effects of another neurotropic RNA virus, the Bornavirus, on the developing brain of rat pups. Information regarding the impact of this viral infection on developing neurons and neural circuitry would lend additional insight (and could raise red flags) regarding the potential neurodevelopmental consequences of other related fetal or neonatal viral infections. Neonatal Lewis rats were infected with Borna Disease Virus (BDV) by innoculation into the right cerebral hemisphere (N=6). Controls (N=6) received PBS. Three week-old rats were sacrificed and brains stained using Rapid Golgi (hemispheres) or Golgi-Cox

(cerebellum) procedures. Dendritic branching and spines were quantitated from coded slides. BDV-infected rat brains tended to show widespread dysmorphic changes in neurons characterized by reduced branching of the dendritic arbor and loss of complexity of the tree, and of dendritic spine loss, often accompanied by dendritic branch varicosities Significant effects of the viral infection were seen in all regions evaluated. The most salient reductions were found were found in: (1) *Cerebellar Purkinje cells:* branching area (-21%), spines (-16%); (2) *Hippocampal CA1s:* basilar branching (-20%), spines (-9%); (3) *Dentate Gyrus Granule cells*: branching (-29%), spines (-25%, thin spines); (4) *Parietal Cortex Layer V pyramids*: basilar branching (-19%), spines (-12%). These findings suggest that early exposure to a neurotropic RNA virus can disrupt maturation of normal dendritic parameters, impair neuroplasticity, and damage brain circuitry. As such, these virus-related dysmorphic changes may represent an underlying neurostructural basis for the pathogenesis of subsequent cognitive impairment and/or developmental disorders.

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Poster

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Topic: A.07. Developmental Disorders

Support: OBI - POND

Ontario Graduate Scholarship

Title: The role of the peripheral immunophenotype in behaviour

Authors: ***S. L. THOMPSON**[1], C. MORI[2], S. PAVALAGANTHARAJAH[2], D. M. E. BOWDISH[3], J. A. FOSTER[1];
[1]Psychiatry and Behavioural Neurosci., [3]Pathology and Mol. Med., [2]McMaster Univ., Hamilton, ON, Canada

Abstract: Recent behavioural studies in our laboratory revealed decreased anxiety-like behaviour in T cell deficient mice. Specifically, mice lacking the β and δ chains of the T cell receptor (*TCRβ-/-δ-/-*) showed reduced anxiety-like behaviour in several approach/avoidance

behavioural tests including the elevated plus maze (EPM), open field test, and light/dark box test in adulthood. Research from our laboratory also indicates the relevance of early life immune function to later behavioural outcomes, as mice challenged with lipopolysaccharide in the first week of life demonstrated sex-specific alteration in both the temporal emergence and phenotypic expression of anxiety-related and exploratory behaviours. This study examines peripheral immune cells and behaviour in adolescence and adulthood in wild type (WT) and *TCRβ-/-δ-/-* mice. Behaviour was assessed using the EPM at postnatal day 28 (P28, pre-puberty in mice), marble burying at P42, open field at P56, novel object at week 10 and 14, and fear conditioning at week 16. Both male and female *TCRβ-/-δ-/-* mice showed increased exploratory behaviour measured by time spent in the intersection zone of the EPM and reduced anxiety-like behaviour measured by increased number of open arm entries. Interestingly, male *TCRβ-/-δ-/-* mice showed increased risk assessment behaviours measured by head dips while on the open arm that was not observed in female *TCRβ-/-δ-/-* mice. No difference was observed in marble burying. Increased locomotor activity was observed in the open field. The behavioural differences observed in T cell deficient mice pre-puberty mirror previous findings in adult mice, suggesting that T cells influence the central nervous system early in development. Genotype and sex-by-genotype effects were observed in both cued and contextual fear conditioning in *TCR β-/-δ-/-* mice. Analysis of novel object is ongoing. Results of FACS analysis at P28 shows increased neutrophil numbers in both male and female *TCR β-/-δ-/-* mice that was significantly associated with behaviour. While additional analysis is ongoing, these initial findings suggest that changes in the profile of immune cells influences anxiety-like behaviour.

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Poster

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Topic: A.07. Developmental Disorders

Support: NSERC

CIHR

Title: Maternal high fat diet affects microglial function in prepubertal immune challenged offspring: Implications for neurodevelopmental disorders

Authors: *M. BORDELEAU[1], M.-E. TREMBLAY[2], G. N. LUHESHI[1];
[1]Douglas Mental Hlth. Univ. Institute, McGill, Verdun, QC, Canada; [2]CRCHUQ Univ. Laval, Québec, QC, Canada

Abstract: Background. Neuropsychiatric disorders, like schizophrenia (SCZ), represent an important burden for the individual and the society. Understandably, a considerable research effort aims to unravel the underlying pathological mechanisms. Recent research showed that the synergistic effects of prenatal and peripubertal stressors lead to behavioral anomalies reminiscent of schizophrenia. This "double hit" model suggests that a prenatal stress could induce brain vulnerability to subsequent stressors. Considering the dramatic increase of high fat diet (HFD) consumption in the West, it is crucial to investigate the repercussions of this biological stressor. HFD consumption is known to increase pro-inflammatory cytokines and, during pregnancy, leads to microglial activation in the offspring. Microglia are involved in synaptic pruning during development suggesting that their abnormal activation could result in neuronal circuit miswiring, leading to abnormal schizophrenia-like behaviors. Objective. Our aim is to understand the effect of maternal HFD, as a prenatal biological stressor, rendering the developing brain vulnerable to subsequent stressors that may lead to SCZ-like pathology through aberrant synaptic pruning. Methods. To test this hypothesis, we used a "double hit" mouse model where maternal high saturated fat diet was used as a prenatal stressor combined with administration of lipopolysaccharide at postnatal day 30 in the offspring, to mimic bacterial infections occurring at puberty. Behavioral paradigms were performed to assess resulting changes in anxiety, stereotyped behaviors, as well as impairment of learning, memory, and social interactions. Microglial function, neuronal connections and neuron-microglia interactions were evaluated in the adult brain (P80) of these offspring using immunohistochemical staining, quantitative-PCR and western blot. Results. Animals from the "double hit" group presented more pronounced behavioral anomalies during adulthood compared to the control and "single hit" groups. These behavioral changes were associated with changes in some of the molecular signals involved in microglial pruning. Conclusion. Our study provides new insights into the mechanisms underlying brain vulnerability to common biological stressors and their deleterious effects on microglial function. In the long-term, it could lead to the development of new therapeutic targets for schizophrenia, as well as new nutritional guidelines for pregnant and nursing women aimed at preventing brain developmental disorders such as SCZ.

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Poster

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UNIBS EX60% 2015-MEMO

Title: NF-κB p50 knock-out mice as animal model of maternal immune activation linked to neurodevelopmental disorders

Authors: *S. A. BONINI, A. MASTINU, M. PREMOLI, G. MACCARINELLI, G. FERRARI-TONINELLI, M. MEMO;
Dept. of Mol. and Translational Med., Univ. of Brescia, Brescia, Italy

Abstract: Introduction: Recently, increasing research has focused on the connections between the immune system and the nervous system, including its possible role in the onset of several neurodevelopmental disorders (NDDs). The hypothesis is that aberrant immune activity during vulnerable and critical periods of neurodevelopment could participate in the generation of neurological dysfunction characteristic of several NDDs, including autism, schizophrenia, and epilepsy. Numerous epidemiological studies have shown an association between maternal infections and the risk of NDDs; animal models of Maternal Immune Activation (MIA) have confirmed this dangerous association. **Aims:** One of the pathway with a main role both in neuron plasticity and immune system regulation is nuclear factor-κB (NF-κB). In this study, we evaluated whether mice lacking the NF-κB p50 subunit ($p50^{-/-}$) represent a new model of MIA and if they present alterations in cortical structure, with consequent behavioural impairments. **Methods:** $p50^{-/-}$ and wild type mice were analyzed, in terms of inflammation, cortical structure and behavioural abnormalities, to clarify the role of a biological mechanism (NF-κB pathway) in the pathophysiology of NDDs and to study possible ways of therapeutic intervention. Experiments were conducted on both male and female mice. **Results:** $p50^{-/-}$ mice have been reported to show multifocal defects in immune responses and we found that they display a chronic inflammation phenotype. Indeed, we observed increased brain gliosis in the cortex and altered cytokines levels in the serum of $p50^{-/-}$ mice. Looking at structural brain alterations, we found that $p50^{-/-}$ mice at post-natal day 2 present an increase in radial glial cells, an in Reelin protein expression levels, other than a specific alteration in the cortical layering. Moreover, adult $p50^{-/-}$ mice display abnormal columnar organization in the somatosensory cortex, a specific decrease in somatostatin- and parvalbumin-expressing interneurons, altered neurite orientation and a concomitant decrease in Synapsin I protein levels. Concerning behaviour, $p50^{-/-}$ mice, other than an increase in locomotor and exploratory activity, present impairments in social behaviours, with a reduction in social interaction, and impaired communication. Finally, we found that Risperidone treatment decreased hyperactivity in $p50^{-/-}$ mice. **Conclusions:** Together, these data provide new insight on the possibility of a link between altered function of NF-κB and the pathogenesis of NDDs. We propose NF-kB $p50^{-/-}$ mice as a new mouse model of MIA leading to NDDs with social impairment.

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UC Davis Behavioral Health Center of Excellence

Title: Poly(I:C) immune response is dependent on length: Implications for preclinical maternal immune activation models

Authors: *M. D. BAUMAN[1], M. CAREAGA[2], C. CHANG[2], A. CHIANG[2], K. KU[2], R. F. BERMAN[2];
[1]Univ. California, Davis, Sacramento, CA; [2]Univ. California, Davis, Davis, CA

Abstract: The Polyinosinic-polycytidylic acid (PolyIC) model, which mimics the acute phase of a viral infection, has become one of the most widely used models in maternal immune activation (MIA) research, particularly for research in the fields of schizophrenia and autism. PolyIC consists of a chain of double stranded inosine (I) and cytidine (C), and as a result of its manufacture the chain length and therefore molecular weight can vary dramatically. Recent studies have shown that *in vitro*, the ability of PolyIC to stimulate an immune response can vary greatly depending on molecular weight. The present studies were carried in order to determine the relationship between molecular weight, immune system activation and sickness behavior. This information is critical for further development of the MIA. Females rats were treated with either saline, low molecular weight PolyIC (LMW-PolyIC), or high molecular weight PolyIC (HMW-PolyIC). Levels of the proinflammatory cytokine IL-6 were measured at 3, 4.5, and 6 hours after injection. Sickness behavior, including core temperature, activity and basal metabolism, was monitored for 24-hours post injection. Mean serum IL-6 was significantly elevated three hours after injection in animals receiving LMW-PolyIC compared with those receiving saline alone ($p < 0.01$). Animals receiving HMW-PolyIC had significantly higher IL-6 levels compared to saline controls ($p < 0.001$), and significantly higher levels over LMW-PolyIC ($p = 0.02$). Similarly, at both 4.5 ($p = 0.006$) and 6 ($p = 0.002$) hours after injection animals receiving HMW-PolyIC had significantly higher levels compared with animals receiving LMW-PolyIC. PolyIC of varying molecular weights can induce significantly different IL-6 responses *in vivo*. This has major implications for the MIA model and highlights the need to validate the reagents used, and to interpret of the results of previous studies with these results in mind.

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Poster

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SFARI

HMM: Postdoctoral fellowship from Child Research Institute at the Stanford Lucile Packard Children's hospital

Title: Toll-like receptor-selective placental vulnerability, fetal brain impairment and post-natal behavioral deficits in models of neurodevelopmental disorder

Authors: *B. A. BABINEAU[1], H. M. MOON[1], A. R. NARAYAN[1], K. M. CORREA[1], V. SARAVANAPANDIAN[1], G. SUBRAMANYAM[3], T. CISNEROS[2], M. RIVERA[1], T. D. PALMER[1];
[1]Dept. of Neurosurg., [2]Immunol. graduate program, Stanford Univ., Stanford, CA; [3]CIRM Bridges Program, Biol. Sciences, Concentration in Mol. Biol. and Microbiology, San Jose State Univ., San Jose, CA

Abstract: Neurodevelopmental disorders (NDD), such as autism and schizophrenia have a diverse and multi-faceted etiology that is poorly understood, though epidemiological studies suggest that environmental risks such as prenatal infections or other gestational immune events correlate with increased NDD risk. Innate immune responses are evoked by toll-like receptor (TLR)-dependent signaling pathways. Both bacterial- (TLR4 selective) and viral- (TLR3-selective) mimetic-mediated maternal immune challenges have been shown to result in brain and behavioral changes (as reviewed by Meyer 2014), however, differences in methodologies prevent the direct comparison of the TLR- selective effects. Previously, we demonstrated that a TLR4-selective insult at E12.5 has adverse effects on fetal and placental health, proliferation of radial glial cells, altered cortical laminar patterning in the adult and behavioral deficits (Carpentier et. al 2013). Here, we aim to directly determine the differential effects of TLR3- and TLR4-selective agonists on a similar set of assays. Placental pathology and pregnancy outcomes were evaluated by quantifying tissue necrosis and fetal survival, respectively. Neocortical alterations in the developing fetuses were examined via immunohistochemistry for markers of cell proliferation and neural progenitor cell populations. Finally, behavioral outcomes were measured using tasks that evaluate behaviors analogous to the symptoms of NDDs, including pup vocalizations, social approach and pre-pulse inhibition. Our results indicate that bacterial and viral immune insults differentially affect placental health, fetal viability and early embryonic

brain development. Post-natal behaviour outcomes also appear divergent with the bacterial insult leading to an earlier onset and more severe phenotype of behavioural alterations. These findings suggest that specific immune events create differential outcomes for the health of the placenta and fetus and lead to distinct changes in cortical patterning which can ultimately manifest as unique behavioural symptoms. Our results have implications for understanding how similar environmental insults may lead to distinct developmental disorders or contribute to the heterogeneity of a specific condition.

Disclosures: **B.A. Babineau:** None. **H.M. Moon:** None. **A.R. Narayan:** None. **K.M. Correa:** None. **V. Saravanapandian:** None. **G. Subramanyam:** None. **T. Cisneros:** None. **M. Rivera:** None. **T.D. Palmer:** None.

Poster

681. Animal Models of Environmental Effects on Neurodevelopment

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 681.11/F17

Topic: A.07. Developmental Disorders

Support: NICHD Grant R15HD092638

Title: Gene by environment interactions in a maternal allergic asthma model of Autism Spectrum Disorders.

Authors: ***F. J. EMERSON**, M. L. BERKOWITZ-CERASANO, J. J. SCHWARTZER; Neurosci. and Behavior, Mount Holyoke Col., South Hadley, MA

Abstract: Autism Spectrum Disorders (ASD) are a class of neurodevelopmental disorders that currently affect around 1 in 68 children in the U.S. While genetics are key factors in the development of ASD, environmental factors such as perturbations *in utero* are believed to play a crucial role. Recent reports suggest an association between maternal allergic asthma (MAA) and a heightened risk of having a child later diagnosed with ASD. However, these studies are limited in identifying causal mechanisms and are unable to control for genetic susceptibility. Mouse strains afford the unique opportunity for exploring how maternal immune alterations may interact with genetic background to exacerbate environmental insults. For example, while both C57 and FVB mice show high social approach behaviors, FVB mice exhibit an enhanced immunological response to allergic asthma exposure compared to C57 mice. Therefore, it was hypothesized that MAA exposure in FVB dams would result in exacerbated ASD-like behavioral responses in offspring compared to C57 mice. To test this, we utilized a novel MAA mouse model in which female mice were sensitized and exposed to repeated allergic asthma inductions

throughout pregnancy. Male and female C57 and FVB offspring were tested for autism-associated behavioral deficits following developmental exposure to maternal allergic asthma. Our findings indicate an interaction between mouse strain and MAA exposure on sociability measures. Interestingly, while C57 mice showed no changes in sociability between treatment conditions, FVB offspring showed altered social approach and reciprocal social interaction when born to MAA-exposed dams, indicating a gene by environment interaction. These findings support a link between maternal allergic priming and altered social development in offspring.

Disclosures: F.J. Emerson: None. **M.L. Berkowitz-Cerasano:** None. **J.J. Schwartzer:** None.

Poster

681. Animal Models of Environmental Effects on Neurodevelopment

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Program#/Poster#: 681.12/F18

Topic: A.07. Developmental Disorders

Support: NIH Grant R15HD082638

Title: ASD-like behavioral deficits in a mouse model of gestational exposure to concentrated ambient particulate matter.

Authors: *M. A. COBURN[1], M. E. JOHNSON[1], P. B. TIJERINA[2], J. ZELIKOFF[2], J. SCHWARTZER[1];
[1]Mount Holyoke Col., South Hadley, MA; [2]Envrn. Med., New York Univ., Tuxedo, NY

Abstract: Exposure to city-level pollution, especially fine-sized particulate matter (<2.5 microns in diameter; $PM_{2.5}$), can have adverse neurodevelopmental effects. For example, epidemiological studies indicate that living in close proximity to a highway is associated with an increased risk of having a child who is later diagnosed with autism or other neurodevelopmental disorders. Mouse models offer a unique opportunity to directly test the biological and behavioral consequences of air pollution and identify which specific constituents produce neurodevelopmental deficits. Pregnant female $B_6C_3F_1$ mice underwent whole-body exposure to Concentrated Ambient $PM_{2.5}$ (CAPs) or Filtered Air (FA) for 6 hours per day throughout gestation followed by additional exposures 2 hours per day for 10 days postpartum. Adult offspring were then tested for autism-associated behavioral changes including reciprocal social interaction, social approach and recognition, and repetitive grooming behavior. Results revealed significant impairments in social behavior and increased repetitive grooming in male mice exposed to CAPs throughout development. These data support the link between environmental air pollution and increased risk of neurodevelopmental disorders.

Disclosures: **M.A. Coburn:** None. **M.E. Johnson:** None. **P.B. Tijerina:** None. **J. Zelikoff:** None. **J. Schwartzer:** None.

Poster

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Program#/Poster#: 681.13/F19

Topic: A.07. Developmental Disorders

Support: NIH Grant R21 NS095351

Title: Deleterious impact of hyperoxia on the development of hippocampus in a mouse model of premature birth injury

Authors: ***C.-M. VACHER**[1,2], J. ABBAH[1], L.-J. CHEW[1], V. GALLO[1];
[1]Children's Natl. Med. Ctr., Washington, DC; [2]Paris Saclay Inst. of Neurosci., Orsay, France

Abstract: At birth, the newborn is exposed to a sudden transition from low oxygen *in utero* to relatively high oxygen tension in the extrauterine environment. With an already underdeveloped antioxidant defense system, coupled with high frequency of oxygen ventilation for lung complications, the preterm infant becomes particularly vulnerable to oxidative stress from reactive oxygen species (ROS). This can lead to cerebral damage and neurological sequelae, including learning difficulties. Adolescents born preterm show spatial memory deficits correlated with a smaller hippocampus[1], a brain structure important for cognitive processing. Disrupted hippocampal development is implicated, but the effects of high oxygen levels on ROS production and its consequences in developing hippocampal neurons have not been established. This study aims to evaluate 1/ the impact of hyperoxia (HO) on ROS production and its consequences in developing hippocampal neurons in a mouse model of neurodevelopmental injury, and 2/ the benefits of a pharmacological intervention by inhibiting GSK3β, a pro-apoptotic and anti-proliferative kinase.
Using a hyperoxia model of perinatal brain injury[2,3], which consists of exposing mice to high oxygen tension (80%) from postnatal day (P) 6 to 8, we have shown that HO induces a significant production of ROS (as evidenced by injecting pups with di-hydroethidium, a cellular superoxide indicator) in all subfields of the hippocampus (CA1-3 and dentate gyrus). This was associated with exacerbated cell death and reduced cell proliferation. By P60, HO mice exhibited a significant loss of interneurons in the whole hippocampus, and the dendritic arborization of remaining interneurons was significantly less complex than room air controls. Furthermore, GSK3β, a kinase known to increase apoptosis and to decrease neurogenesis and neuritogenesis, was more active in the hippocampus following HO. Finally, the HO-induced cellular alterations

observed at P8 and P60 were entirely prevented by GSK3β inhibitor administration during HO exposure.
In conclusion, our data show that early-life exposure to HO leads to increased hippocampal levels of ROS and the activated form of GSK3β. This is accompanied by a decline in neurogenesis and a disrupted development of the local inhibitory network. Taken together, these modifications may account for the diminished learning ability observed in our mouse model as well as in humans. Notably, the inhibition of GSK3β could represent a potential therapeutic intervention against the neurological outcomes of preterm birth.
[1] Isaacs et al, Pediatric Res, 2000; [2] Schmitz et al, J Neuroscience, 2011; [3] Ritter et al, J Neuroscience, 2013.

Disclosures: **C. Vacher:** None. **J. Abbah:** None. **L. Chew:** None. **V. Gallo:** None.

Poster

681. Animal Models of Environmental Effects on Neurodevelopment

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Topic: A.07. Developmental Disorders

Support: CDER FDA

NCTR FDA

Title: Long-term cognitive dysfunction associated with isoflurane/nitrous oxide-induced general anesthesia in neonatal nonhuman primates is greatly diminished by prophylactic administration of acetyl-l-carnitine

Authors: ***M. G. PAULE**[1], M. LI[1], S. LIU[1], X. ZHANG[1], J. P. HANIG[2], W. SLIKKER, Jr.[1], C. WANG[1];
[1]Div. of Neurotoxicology, FDA's Natl. Ctr. For Toxicological Res., Jefferson, AR; [2]Office of Testing and Res., Ctr. for Drug Evaluation and Res., Silver Spring, MD

Abstract: In proof-of-concept studies in rhesus monkeys it was shown that 24 hours of ketamine-induced general anesthesia during the first week of life caused significant neuronal cell death and seemingly permanent cognitive deficits in rhesus monkeys. In subsequent studies similar effects were noted after only 8 hours of general anesthesia induced by isoflurane (ISO, 1%) plus nitrous oxide (N2O, 70%). In both cases, subjects began training at 7 months of age to perform cognitive function tasks as part of the National Center for Toxicological Research Operant Test Battery (OTB): these included tasks for assessing learning, motivation, color

discrimination, and short-term memory. Subjects responded for food pellets by pressing response levers and press-plates during daily (M-F) test sessions (50 min). Training scores were assigned based upon their individual task performance. Beginning as early as 8 months of age—and continuing throughout prolonged testing (>2 years) typically with no indication of recovery—exposed animals earned fewer reinforcers in a task assessing appetitive motivation, completed less of a task designed to assess daily learning, and performed a visual discrimination tasks less efficiently than controls. Acetyl-l-carnitine, an anti-oxidant and mitochondrial membrane stabilizer, when given prophylactically was able to prevent many of the adverse functional effects of the ISO plus N2O anesthesia. These data suggest that specific behavioral phenotypes are associated with general anesthetic exposures that occur during a sensitive period of brain development and that certain agents will be able to ameliorate at least some of the developmental neurotoxicity associated with general anesthesia.

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Poster

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Topic: A.07. Developmental Disorders

Title: Ketamine anesthesia during the first week of life can cause permanent cognitive deficits in rhesus monkeys

Authors: *J. C. TALPOS, III, J. CHELONIS, M. LI, M. P. GILLAM, C. WANG, W. SLIKKER, M. G. PAULE;
Natl. Ctr. for Toxicological Res., FDA, Jefferson, AR

Abstract: As a result of advances in medical practice more children born pre-term or with birth complications are surviving to adulthood. An unintended consequence of this is that more children are being exposed to various anesthetic and sedative agents at increasingly younger ages. The developmental consequences of these exposures on cognitive function are largely unknown because it is difficult to evaluate the safety of these agents directly in a pediatric population. In this circumstance nonhuman primates serve as necessary alternatives to estimating the neurotoxicological risk associated with perinatal drug exposure in very young children. In an attempt to model human pediatric anesthetic use, we exposed rhesus monkeys on post-natal day 5 or 6 to intravenous ketamine-induced general anesthesia (24h). When these monkeys reached 7 months of age, we started training them on the National Center for Toxicological Research

(NCTR) Operant Test Battery (OTB) to determine potential effects on cognitive ability. The OTB is designed to measure various aspects of complex brain function including color and position discrimination, working memory, motivation, and temporal discrimination / impulsivity and learning. We previously reported that after nearly 3 years of training animals exposed to ketamine performed worse on a color and position discrimination task and on a progressive ratio (PR) motivation task. These animals, now adults 9-10 years old, continue to show a similar pattern of impairment when compared to a control group (n=6). The ketamine group (n=6) consistently performs worse on an incremental repeated acquisition task (spatial discrimination learning task), earns fewer rewards in a temporal response discrimination (timing) task and a PR task and responds more slowly. Over the last 100 sessions the ketamine group has on average performed 1 standard deviation below the control group on these measures, suggesting that these impairments are permanent in the absence of a remediation program. Yet some areas of function appear to be preserved: no impairment was observed in the ability to learn a visual conditioned place response *after* response contingencies were reversed. These data indicate that 24h of ketamine anesthesia on postnatal day 5 or 6 is capable of causing permanent changes in cognitive performance in the rhesus monkey and suggest that a similar ketamine general anesthetic regimen would likely have detrimental consequences in very young human infants. Additional work is underway to determine if this effect is specific to ketamine, or a more general consequence of neonatal anesthesia.
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Poster

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Topic: A.07. Developmental Disorders

Support: NIMH Grant R01-MH087583

NIMH Grant R01-MH099085

NIMH Grant R01-MH058616

Title: Prenatal valproic acid exposure induces sex specific autism-related behavioral phenotypes in prairie voles

Authors: *L. L. ELVIR[1], H. WANG[2], F. DUCLOT[1], Y. LIU[2], Z. WANG[2], M. KABBAJ[1]; [1]Biomed. Sci., [2]Psychology, Florida State Univ., Tallahassee, FL

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. Although significant discoveries on the embryopathology of VPA have been proposed, not one study has assessed its effects on social bonding, a complex behavior not exhibited by rats and mice. In this study, we aimed at validating the socially monogamous prairie vole (*Microtus ochrogaster*) model for the study of the effects of prenatal VPA exposure. Male and female control and VPA-prenatally exposed subjects were assessed on a battery of behavioral 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. An interesting sex difference is found: VPA-pretreated male, but not female, subjects showed enhanced anxiety-like behavior and did not develop partner preference during adulthood, in the absence of mating. We are now in the process of examining if partner preference formation is disrupted following cohabitation with mating in response to prenatal exposure to VPA. We are also examining, in the prefrontal cortex, mRNA and protein expression of genes that modulate social bonding in prairie voles, such as the oxytocin and vasopressin receptors, as well as genes largely implicated in neurodevelopmental disorders and involved in synaptic formation and signaling, such as Shank3, Nlgn3, and MeCP2.

Disclosures: L.L. Elvir: None. **H. Wang:** None. **F. Duclot:** None. **Y. Liu:** None. **Z. Wang:** None. **M. Kabbaj:** None.

Poster

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Topic: A.07. Developmental Disorders

Support: T32DA007262-25

MH096773

UL1TR000128

Title: Chronically elevated prenatal cytokine exposure as a translational model for ADHD and ASD. Rodent behavior and resting state connectivity MRI

Authors: *B. D. MILLS[1], A. SHUMNMUGAVEL[2], A. GONCHAROVA[2], C. PIZZIMENTI[2], M. LATTAL[2], S. MITCHELL[2], D. FAIR[2];
[1]Oregon Hlth. & Sci. Univ., Portland, OR; [2]Behavioral Neurosci., Oregon Hlth. and Sci. Univ., Portland, OR

Abstract: Attention deficit hyperactivity disorder (ADHD) and autism spectrum disorder (ASD) have been linked to prenatal risk factors including maternal obesity, stress, and viral infections during pregnancy. These risk factors share a common mechanism, elevated inflammatory cytokines and in particular elevated interlukin-6 (IL-6). Resting state functional connectivity (rs-fcMRI) abnormalities have been identified in both ADHD and ASD and have similarly been identified in infants whose mothers had elevated IL-6 blood levels. The current work uses a rodent model to test the causal mechanisms of IL-6 exposure and behavioral and neural phenotypes associated with these developmental disorders. Sprague-Dawley rats were implanted with osmotic pumps delivering either saline or a daily dose 4.98 ug/kg IL-6 over the course of 40 days. This dose was designed to mimic chronic inflammatory states throughout pregnancy. Offspring were given a battery of behavioral tests and rsfc-MRI imaging both early (PD25) and late (PD50) in development. Here, we find that IL-6 exposed offspring show a robust anxiety phenotype on both the open field and light dark behavioral tests. We also found that IL-6 animals more readily acquired fear, as measured by increased freezing to three .5 mv footshocks. No differences in social approach behavior was found. Finally, to serve a translational tool, we discuss atypical network structure, default mode network connectivity in IL-6 exposed animals, developmental change in rs-fcMRI, and the relationship of these findings to connectivity structure seen in developmental disorders. Overall, this work sheds light on the underlying mechanisms associated maternal inflammation and the role of IL-6 as risk factor for developing phenotypes seen in ASD and ADHD.

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Poster

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Topic: A.07. Developmental Disorders

Support: NIH NINDS PO1NS083513

Title: Developing a ferret model of newborn brain injury and cortical dismaturation

Authors: A. MIKHAILOVA[1], J. K. ELLIS[2], *P. S. MCQUILLEN[3];
[1]Pediatrics, [2]Regeneration Med., [3]Pediatrics and Neurol., Univ. of California San Francisco, San Francisco, CA

Abstract: Background: Existing small animal models fail to reproduce permanent dysmyelination 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. Objective: 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. Methods: Using a previously described method of chronic sublethal hypoxia (Tao et al Pediatric Research (2012) 71, 192-198), jill and kits are exposed to 10% oxygen from P10-20. Some kits are examined at P20, while the rest are reared in normoxia until sacrifice. Age matched controls are reared in normoxia. Results: (1) Consistent with published findings, following exposure to hypoxia, myelin basic protein expression decreases and glial fibrillary protein expression increases at P20 and P30. We evaluated the persistence of these changes into later ages - at P40, GFAP is still upregulated in white matter, and MBP doesn't extend out into lower cortical layers like in control (normoxic) animals. Studies are in progress to determine the permanency of changes and evaluate functional myelination (e.g. electron microscopy). (2) Using cell proliferation markers we find decreased density of ki67 positive cells at P20 following hypoxia vs age matched control, but ki67 density is greater at P30 following hypoxia than age matched control. We are using neural cell lineage-specific markers to determine which cells undergo changes in proliferation and potential associated maturation arrest. (3) Hypoxia during this period (P10-20) also influences newly identified postnatal neuronal migration streams, which are most active at P40. Following hypoxia, the transcription factor Sp8 is increased in the white matter and its laminar organization is affected in the cortex. Future analysis will look at younger ages (P20, P30) to evaluate the progression of Sp8 expression. Lastly, we are using lineage tracing techniques to track the postnatal neuronal migration in normal development and following hypoxia. Conclusion: 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.

Disclosures: A. Mikhailova: None. **J.K. Ellis:** None. **P.S. McQuillen:** None.

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Topic: A.07. Developmental Disorders

Support: TIFR Intramural Grant

Title: Postnatal fluoxetine treatment evokes persistent alterations in perineuronal nets within the hippocampus

Authors: S. MUKHOPADHYAY[1], A. SOOD[1], *V. A. VAIDYA[2];
[1]Tata Inst. Fundamental Res., Mumbai, India; [2]Tata Inst. Fundamental Rese, Mumbai, India

Abstract: Major depressive disorder is a complex and widespread psychiatric disorder. Studies have indicated that early life events have a powerful role in modulating the development of emotional neurocircuitry, and that fine tuning of these circuits is greatly influenced by serotonergic signaling in this period. Increasing availability of synaptic serotonin in an early postnatal window in rats using a Selective Serotonin Reuptake Inhibitor (SSRI) - Fluoxetine, results in the emergence of depressive- and anxiety-like behaviours in adulthood. We used this postnatal Fluoxetine model of depression (PNFLX) and investigated the acute and long lasting consequences of PNFLX treatment on cellular architecture in key neurocircuits implicated in emotional regulation, focusing on GABAergic interneurons. We studied three non-overlapping subtypes of GABAergic interneurons characterized by the presence of Parvalbumin (PV), Somatostatin (SST) and Calretinin (CR) in the hippocampus and the prefrontal cortex (PFC). Numbers of GABAergic neurons have been shown to be altered in different models of psychopathology, and are especially vulnerable to early life interventions since they continue to migrate and integrate into circuits in this postnatal period. Our results indicate no striking differences in the numbers of PV, SST and CR positive interneurons, in either the hippocampus or the PFC, between the Vehicle treated and PNFLX treated animals in adulthood or immediately after the drug treatment is ceased at postnatal day 21. Further, we studied perineuronal nets (PNNs), which are ECM-associated structures found around fast spiking PV positive interneurons, and play an important role in circuit maturation and stabilization. We observed a reduction in the number of PNNs in the hippocampal subfields of adult animals with a history of PNFLX. This may imply a circuit maturation defect in these PNFLX treated animals arising due to altered serotonergic signaling in a critical window important for the sculpting of emotional neurocircuitry. At least one study has reported that prenatal fluoxetine exposure can hamper formation of hippocampal PNNs, and Fluoxetine administration is known to reopen critical window-like neuronal plasticity in adulthood. We thus hypothesize that increased serotonergic drive as a result of Fluoxetine exposure during an early postnatal window leads to a

defect in the initiation, regulation or closure of the maturation process of emotional neurocircuitry, and that this may underlie at least some of the behavioural deficits observed in this model of early life pharmacological perturbation leading to development of depression and anxiety like behaviours.

Disclosures: **S. Mukhopadhyay:** None. **A. Sood:** None. **V.A. Vaidya:** None.

Poster

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Topic: A.07. Developmental Disorders

Support: CONACyT 181334

Title: Early changes in behavior and brain metabolism in socially isolated rats

Authors: *J. HERNANDEZ-FALCON[1], M. PORRAS-VILLALOBOS[2], E. AGUIRRE-BENÍTEZ[2], L. PARRA-GÁMEZ[2], J. GONZÁLEZ-RÍOS[2], M. AVILA-RODRÍGUEZ[2], D. ALBORES-GARCÍA[2], K. MENDOZA-ANGELES[2], A. MELO[3];
[1]Univ. Nacional Autónoma De México, Ciudad DE Mexico, Mexico; [2]Univ. Nacional Autónoma de México, México, Mexico; [3]Univ. Autónoma de Tlaxcala, Méxco, Mexico

Abstract: Social and sensory experience is vital for a harmonic development, maturation and whole individual organization. Development involves the coordinated growth and functioning of cardiovascular, nervous and endocrine systems, among others. In altricial species, maternal care, immediately after birth and until weaning, determines a well growth in pupae. Social interaction of the cub with its mother and littermates seems determinant for a successful development, such that early social isolation impairs postnatal development.
The main goal of this work was to analyze the effects of social isolation in young artificially reared rats upon behavior, brain glucose metabolism, and astrocyte population in the brain. Three day-old male pups were artificially reared in isolation. Sensory stimulation during artificial rearing was provided each 4 hours. After weaning, we tested animals in the open field paradigm and measured brain use of 18-fluoro-glucose (through micro-PET). After sacrifice, we analyzed astrocyte population in different brain areas.
Results show an erratic behavior in experimental animals: long lasting periods in the open field combined with an increased tendency to bury themselves; higher levels of aggressiveness in the presence of littermates, and increased grooming. PET images showed decreased capture of 18-fluoro-glucose in amygdala and brain cortex. This decrease was more evident during social

interaction with littermates. Astrocytic distribution was also different in experimental animals. In them, we detected a greater number of astrocytes in the same regions. These results indicate that social isolation disturbs brain metabolism and development which is reflected in an altered behavior.

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Poster

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Topic: A.07. Developmental Disorders

Support: Brain & Behavior Foundation (NARSAD)

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Title: Effect of developmental sleep fragmentation on social behavior and parvalbumin expression in prairie voles

Authors: ***M. M. LIM**[1,2,3], E. A. D. HAMMOCK[4], J. QUINTANA[4], R. D. CHAMPAIGNE[1], R. A. OPEL[1], D. L. COCKING[1], R. C. DRIESSEN[1];
[1]Veterans Affairs Portland Hlth. Care Syst., Portland, OR; [2]Behavioral Neuroscience, Medicine, and Neurol., [3]Oregon Inst. of Occup. Hlth. Sci., Oregon Hlth. & Sci. Univ., Portland, OR; [4]Psychology, Florida State Univ., Tallahassee, FL

Abstract: Consolidated sleep periods during development, especially rapid eye movement (REM) sleep, are critical for parvalbumin inhibitory interneuron expression in basic brain circuits (such as the visual system). Parvalbumin expression is significantly decreased in brains of human subjects with autism spectrum disorder. It is unknown whether REM sleep plays a role in the maturation of more complex circuits for social behavior, such as those relevant to autism. 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 behavior. We selectively suppressed REM sleep in prairie vole pups during a sensitive post-natal period of development from P14 to P21. Following developmental sleep fragmentation, voles underwent tests for social behavior as juveniles, and again as adults. At 5 weeks of age, there were no significant differences in social behavior between groups. At 12 weeks of age, male prairie voles showed hyperactive approach behavior towards juveniles, where as females showed decrease approach behaviors. Social memory as assessed by a habituation-dishabituation paradigm did not seem to be affected by sleep fragmentation. However, sleep-fragmented male prairie voles showed profoundly impaired pair bond formation as assessed by the partner preference test. Sleep-fragmented females showed mildly decreased partner preference but significantly increased aggression towards strange males. Locomotor activity assays and wheel running showed that sleep-fragmented males were more hyperactive than controls. Parvalbumin immunohistochemistry was performed to determine if sleep fragmentation disrupted parvalbumin immunoreactive cell counts in the neocortex, and if effects of sleep fragmentation on parvalbumin interneuron are sex or age dependent.
Our data suggest that sleep fragmentation during the third post-natal week profoundly impairs social development. 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.

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Poster

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Topic: A.07. Developmental Disorders

Title: Effects of exposure during adolescence to different PCB aroclors and doses on hyperactivity and impulsiveness in male and female rats

Authors: *J. P. LOMBARDO[1], J. A. PECK[1], D. F. BERGER[2], P. M. JEFFERS[3];
[2]Psychology, [3]Chem., [1]SUNY Col, Cortland, Cortland, NY

Abstract: Previous studies of PCB-induced hyperactivity and impulsiveness in rats suggest that exposure to PCBs have different effects on males and females. To further investigate this possible sex difference, two studies were conducted to examine the effects ingested polychlorinated biphenyls (PCBs) would have on the operant behavior of water deprived

Sprague-Dawley rats. All animals were dipper - trained to bar press for water. All animals were deprived of water for 22-hr prior to test days. In Experiment 1 the diet of adolescent male and female rats was augmented with 0.5 µg/g Aroclor 1248 for 30 days. In Experiment 2, groups of male and female adolescent rats had their diets augmented with 1.5 or 4.0 µg/g Aroclor 1248 for 30 days. Other groups of adolescent male and female rats had their diets augmented with 1.5 or 4.0 µg/g of a 1:1 mixture of Aroclors 1254/1260. In both experiments animals were tested in a mult 120-s FI- 5 min extinction procedure. Exposure to PCBs, in all rats, took place between PND 35-64. On test days, operant lever responding was reinforced with water. In Experiment 1 ingestion of Aroclor 1248 did not produce hyperactivity or impulsiveness in the female rats, but did produce both behaviors in males. Results of Experiment 2 revealed a similar pattern: ingestion of both Aroclor mixtures did not produce hyperactivity in females, but male rats exposed to either Aroclors showed hyperactive and impulsive behavior. Females in Experiment 2 that were exposed either 1.5 or 4.0 µg/g of Aroclor 1248 or the 1254/1266 mixture were *hypoactive* relative to their controls.

Disclosures: J.P. Lombardo: None. **J.A. Peck:** None. **D.F. Berger:** None. **P.M. Jeffers:** None.

Poster

681. Animal Models of Environmental Effects on Neurodevelopment

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 681.23/F29

Topic: A.07. Developmental Disorders

Support: NSERC

AIHS

Title: The impact of preconception paternal experience on offspring neurodevelopment and behavior

Authors: *A. F. HARKER[1], R. DOMBOWSKY[1], B. KOLB[2], R. GIBB[2];
[2]Neurosci., [1]Univ. of Lethbridge, Lethbridge, AB, Canada

Abstract: A rich literature has been amassed demonstrating the impact of early life events on the structure and function of the developing brain. While a plethora of research has shown that maternal experience during the prenatal period of life has the ability to alter neurodevelopment and behavioural outcomes of offspring, far less in understood regarding the impact of preconception paternal experience on developing brain architecture. The goal of this research

was to examine the effect of two independent preconception paternal experiences on subsequent neurodevelopment and behaviour of male and female offspring. Research has shown that stress during the prenatal period can alter brain morphology in the developing brain, and is thought to be a factor in the development of some adult psychopathologies. Our first experiment examined the impact of preconception paternal stress on offspring brain and behaviour. Our hypothesis was that paternal stress in the preconception period would negatively impact brain development in offspring, leading to behavioural abnormalities. While stress and environmental enrichment have been shown to have opposing effects on brain architecture and behavioural outcomes in offspring, we decided to explore the impact of environmental enrichment provided to fathers during the preconception period. We hypothesized that preconception paternal enrichment would alter brain development and positively impact behaviour of offspring. Both experiments followed the same experimental design. Male Long Evans rats were exposed to either a stressing paradigm or a complex environment for 27 days prior to mating. Developmental assays, anatomical measurements, and brain morphology analyses were conducted throughout offspring lifespan.

Disclosures: **A.F. Harker:** None. **R. Dombowsky:** None. **B. Kolb:** None. **R. Gibb:** None.

Poster

681. Animal Models of Environmental Effects on Neurodevelopment

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 681.24/F30

Topic: A.07. Developmental Disorders

Support: NIAAA

Title: Transgenerational effects of prenatal ethanol exposure in a mouse model

Authors: ***D. J. ROHAC**[1], R. NAIR[2], K. J. HUFFMAN[1];
[1]Psychology, [2]Col. of Natural and Agr. Sci., Univ. of California Riverside, Riverside, CA

Abstract: Fetal Alcohol Spectrum Disorders (FASD) describe a range of phenotypes in children whose mothers consumed alcohol during pregnancy. Recent work by our laboratory suggests that prenatal ethanol exposure, or PrEE, alters DNA methylation, one of the main pathways involved with epigenetic inheritance, in the offspring. Thus, we have proposed that some of the hallmark phenotypes of FASD may be heritable to non-exposed generations. To explore how PrEE may induce heritable, transgenerational effects on neocortical anatomy and behavior, we used a murine FASD model in CD-1 mice. Our initial studies on the first filial (F1) generation of PrEE mice found disruptions in the intraneocortical network, changes to the anatomy of the neocortex and altered behavior (El Shawa et al., 2013). In this study, we extend the anatomical and

behavioral analyses in F1 PrEE mice to F2 and F3 generations. If changes are seen in the F3 generation, we can deduce that an epigenetic pathway has been altered and the changes are not caused by direct exposure. Analyses in F2 and F3 PrEE mice were performed on the day of birth (P0) and 20 days later, at normal weaning age (P20). Assessments at P0 included structural thickness measurements in different cortical regions including frontal cortex and the corpus callosum as well as intraneocortical connectivity. We found differences in anatomical structures that persist through the third generation after PrEE and found that results from behavioral assays, measured at P20, demonstrated a heritable phenotype. Specifically: F2 and F3 PrEE mice showed ectopic connections in frontal cortex, altered cortical thickness measures at P0 as well as reduced sensorimotor integration, motor coordination and increased anxiety when assessed on the Suok test at P20. Along with the changes to DNA methylation (Abbott et al., in preparation), these data point to a strong heritable effect of prenatal ethanol exposure that is transmitted via an epigenetic pathway.

Disclosures: D.J. Rohac: None. **R. Nair:** None. **K.J. Huffman:** None.

Poster

681. Animal Models of Environmental Effects on Neurodevelopment

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 681.25/F31

Topic: A.07. Developmental Disorders

Support: NIAAA 1 P50 AA022534

Title: Negative synergism between prenatal alcohol exposure and chorioamnionitis: a preclinical investigation

Authors: *J. MAXWELL, S. DAVIES, D. SAVAGE, L. JANTZIE, PhD;
Univ. of New Mexico Hosp., Albuquerque, NM

Abstract: To identify and refine targets for new therapeutic interventions, a clearer understanding of how *in utero* insults cause impaired neurodevelopment is imperative. Prenatal alcohol exposure (PAE) and placental insufficiency are clinically related, but the mechanisms leading to subsequent brain injury have not been studied in validated animal models. Women who abuse alcohol during pregnancy have a five-fold increased rate of chorioamnionitis, the chief placental abnormality associated with preterm birth and encephalopathy of prematurity. Indeed, numerous structural and diffusion cerebral abnormalities are seen in children with prenatal alcohol exposure. Here, we hypothesized that PAE would exacerbate chorioamnionitis worsening brain injury, including significant deficits in myelination and white matter

microstructure. To this end, pregnant Long-Evans rats were allowed to drink 5% ethanol or saccharin until embryonic day 17 (E17) to mimic moderate PAE. Subsequently, on E19 a laparotomy was performed and the uterine arteries were clamped for 30 minutes to induce placental insufficiency and transient systemic hypoxia-ischemia (TSHI). Lipopolysaccharide (LPS, 4μg) was then injected in to each amniotic sac. The laparotomy was closed and pups born at E22. Pups matured with their dams until postnatal day 15 (P15) and P28 at which time brain tissue was collected. PAE+TSHI+LPS resulted in a severe injury concomitant with significantly increased fetal mortality and reduced postnatal survival ($p<0.05$). Specifically, the combination resulted in 95% fetal mortality and death of live born offspring by P2 (n=5 dams). This fetal and postnatal mortality was significantly greater than that observed in either PAE (3% mortality; n=4 dams) or TSHI+LPS (57% mortality; n=5 dams) alone. Animals with TSHI+LPS alone had reduced white matter fractional anisotropy and impaired axial and radial diffusion compared to sham ($p<0.05$). This is the first report that the combination of prenatal alcohol exposure and chorioamnionitis results in a severe in utero insult consistent with spontaneous abortion in rodents and injury incompatible postnatal life. Future studies will dissect the mechanisms of increased mortality with specific focus on the maternal-placental axis and developing brain. Preclinical models recapitulating prenatal alcohol exposure in the setting of chorioamnionitis will aid in the identification of interventions that may be used to reduce brain injury and chronic neurologic disabilities in children.

Disclosures: **J. Maxwell:** None. **S. Davies:** None. **D. Savage:** None. **L. Jantzie:** None.

Poster

681. Animal Models of Environmental Effects on Neurodevelopment

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 681.26/F32

Topic: A.07. Developmental Disorders

Support: NIH Grant AA019462

NIH Grant 2T32AA014127-11

Title: Differential spatial extinction behavior in male and female rats prenatally exposed to moderate levels of ethanol

Authors: ***C. M. MAGCALAS**[1], J. L. WAGNER[2], S. DAVIES[2], D. D. SAVAGE[2], D. A. HAMILTON[3];
[2]Neurosciences, [3]Psychology and Neurosciences, [1]Univ. of New Mexico, Albuquerque, NM

Abstract: Prenatal alcohol exposure (PAE) is associated with structural and physiological changes that impact the central nervous system and can result in persistent negative consequences in a broad spectrum of cognitive and behavioral domains including deficits in motor behavior, social behavior, and behavioral flexibility. Previous studies have characterized the influence of PAE on spatial navigation acquisition and extinction through various behavioral paradigms including the Morris water task (MWT). The current study focuses on examining the behavioral consequences of PAE on spatial extinction behavior through the use of the MWT. Pregnant rat dams voluntarily consumed saccharin (SAC) water containing 0% or 5% ethanol (EtOH) for 4 hours per day during the entire gestational period. Male and female pups matured and were tested in the MWT in adulthood (>90 days old). In order to assess extinction behavior the animals were tested in a 5-day hidden platform protocol. Days 1-4 of the hidden protocol consisted of 12 training trials. At the end of day 4, the animals were tested in 3 consecutive no-platform probe trials to assess extinction behavior. Day 5 began with one no-platform probe trial to assess spontaneous recovery followed by 12 retraining trials. All of the animals successfully learned the hidden platform goal location during the initial training period. All of the male animals (PAE and controls) failed to extinguish, which was evident by the consistent short latency to reach the learned target location. The control females successfully extinguished, but the ethanol exposed females failed to extinguish the learned behavior. These outcomes suggest that animals exposed to moderate levels of ethanol during gestation have intact spatial acquisition abilities, but may have distinct extinction behaviors that may be sex specific and can be influenced by PAE. [Supported by grant AA019462 to DH].

Disclosures: **C.M. Magcalas:** None. **J.L. Wagner:** None. **S. Davies:** None. **D.D. Savage:** None. **D.A. Hamilton:** None.

Poster

681. Animal Models of Environmental Effects on Neurodevelopment

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 681.27/F33

Topic: A.07. Developmental Disorders

Support: NIH AA019462

Title: Microstructural connectivity of the corpus callosum after moderate prenatal alcohol exposure in the adult rat.

Authors: *C. I. RODRIGUEZ[1], G. LIEBERMAN[1], S. DAVIES[2], D. SAVAGE[2], D. HAMILTON[1];
[1]Psychology, [2]Neurosciences, The Univ. of New Mexico, Albuquerque, NM

Abstract: Previous research revealed microstructural abnormalities in the corpus callosum of individuals exposed to prenatal alcohol (PAE). Subsequent research conducted in a preclinical models of PAE demonstrated similar alterations in several white matter structures including the corpus callosum. However, the effects of moderate exposure on microstructural connectivity of the corpus callosum have not been thoroughly examined. Here, we assessed the effects of moderate PAE on measures of fractional anisotropy (FA), diffusivity, and size of the rat corpus callosum.

Long-Evans rats were exposed to 5% ethanol or saccharin throughout gestation. In adulthood, rats were anesthetized and placed in a 4.7T Bruker Biospin magnetic resonance scanner for the acquisition of diffusion weighted images.

Following image preprocessing, measures of FA, axial diffusivity (AD), radial diffusivity (RD), mean diffusivity (AD), and area were gathered and compared across sex and prenatal treatment conditions by a two-way univariate analysis of variance. No significant interactions for measures of FA, AD, RD, MD, and total sampled area were detected. Significant sex differences for FA, RD, and MD were observed. A significance trend for axial diffusivity was observed for sex. Strong effect sizes were observed for sex differences in measures of FA, RD, and MD. No significant effect of total area sampled for males or females was detected. No significant differences in prenatal treatment condition separated by sex were found when comparing measures of FA, MD, RD, AD, and sampled area.

In contrast to previous research conducted on children and adults with documented PAE, these results suggest that moderate PAE does not negatively impact axonal organization of the corpus callosum in a preclinical model of PAE. Additional reports of FA in preclinical models are mixed and implicate FA as an unreliable marker for PAE. Consistent with these findings, is a report that points to deficits in white matter myelination, but intact axonal integrity, after a single binge-like exposure to alcohol on gestational day (GD) 7. Moreover, measures of anisotropy, diffusivity, and area were limited to the whole coronal sections of corpus callosum as opposed to sagittal sections that have revealed regional differences in posterior regions of the corpus callosum. As a result, additional work is needed to examine regional differences in the corpus callosum. Additional methods that are more sensitive to white matter myelination merit strong consideration. The present findings add to the body of literature emphasizing functional and behavioral consequences of PAE may occur in the absence of structural abnormalities.

Disclosures: C.I. Rodriguez: None. **G. Lieberman:** None. **S. Davies:** None. **D. Savage:** None. **D. Hamilton:** None.

Poster

681. Animal Models of Environmental Effects on Neurodevelopment

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 681.28/F34

Topic: C.09. Brain Injury and Trauma

Support: NIH/NIGMS COBRE: The Delaware Center for Neuroscience Research grant 1P20GM103653 - 01A1 to AYK.

Title: Differential impact of exercise and environmental complexity on hippocampal Bdnf DNA methylation, gene expression, and dendritic complexity in a rat model of FASD

Authors: *A. Y. KLINTSOVA, K. E. BOSCHEN, T. L. ROTH;
Psychological and Brain Sci., Univ. of Delaware, Newark, DE

Abstract: Exposure to ethanol *in utero* in humans results in neuroanatomical, cognitive, behavioral, and physiological deficits collectively known as Fetal Alcohol Spectrum Disorders (FASD). Third trimester-equivalent (postnatal days [PD] 4-9) alcohol exposure (AE) in a rat model negatively impacts hippocampal neuroplasticity, increases apoptosis, and impairs adult neurogenesis and LTP. The current study investigates Brain-derived Neurotrophic Factor (*Bdnf*) gene expression, *Bdnf* DNA methylation, and granule cell dendritic complexity in the adult rat hippocampus following neonatal AE. Previous work from our lab has shown that PD4-9 AE increases BDNF and TrkB protein and *Bdnf* gene expression in the PD10 hippocampus (Boschen et al., 2015). Additionally, our model of FASD alters dendritic morphology in medial prefrontal cortex on PD72. For the current study, male AE pups received 5.25 g/kg/day of alcohol on PD4-9 in a binge-like manner via intragastric intubation. Two control groups were used: sham-intubated (SI) and suckle control (SC). From PD30-72, rats were housed in one of three conditions: standard housing (SH; 3/cage), voluntary wheel running (WRWR; 3/cage), or wheel running (PD30-42) followed by housing in a complex environment (PD42-72; 9-12/cage) (WREC). Tissue from the hippocampus was collected on PD72. AE animals had decreased dendritic complexity of immature (doublecortin/DCX-positive) dentate gyrus neurons on PD72, and these alterations to dendritic morphology were rescued by WREC and WRWR. No baseline *Bdnf* gene expression changes were found at PD72 between neonatal conditions. Interestingly, WRWR significantly increased gene expression in all three neonatal treatment groups, however WREC did not alter levels of *Bdnf* mRNA. For *Bdnf* DNA methylation, preliminary data suggests no changes to steady-state methylation status in AE animals, and lower methylation following access to WRWR. Overall, these data indicate that while neonatal alcohol exposure has pronounced effects on *Bdnf* gene expression shortly following exposure (Boschen et al., 2015), these alterations become more subtle across the lifespan. In addition, this work shows that our model of FASD negatively impacts granule cell dendritic complexity independent of

hippocampus-wide alterations to basal *Bdnf* expression. Finally, our data support the further investigation of WREC and WRWR as therapeutic interventions for the alcohol-damaged brain.

Disclosures: **A.Y. Klintsova:** None. **K.E. Boschen:** None. **T.L. Roth:** None.

Poster

681. Animal Models of Environmental Effects on Neurodevelopment

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 681.29/F35

Topic: F.03. Neuroendocrine Processes

Support: R01 AA11591

Title: A canonical Wnt-Frizzled pathway regulates epithelial mesenchymal transition in prolactin-secreting tumors in the pituitary of fetal alcohol exposed rats

Authors: S. JABBAR[1], W. BELDEN[2], J. PARK[2], *D. K. SARKAR[2];
[1]Animal Sci., Rutgers Univ., New Brunswick, NJ; [2]Rutgers, SUNJ, New Brunswick, NJ

Abstract: Prolactin-secreting pituitary tumors (prolactinomas) are the most common pituitary tumors in humans. Majority of prolactinomas are adenomas and benign and slow growing, but in some cases, they are locally aggressive and invasive. We have recently shown that alcohol abuse during pregnancy result in fetal programming of the pituitary gland to produce aggressive prolactin-producing tumors in the offspring. In this study, we employed RNA-seq to identify putative genes responsible for tumor aggressiveness in alcohol-programmed pituitaries. Pregnant Fischer 344 rats were fed between gestational days 7 and 21 with a liquid diet containing 6.7 % alcohol (AF), pair-fed with isocaloric liquid diet (PF), or fed ad libitum with rat chow (AD). At 60 days of age, female offspring rats were ovariectomized and received a subcutaneous estradiol implant. These rats were sacrificed at 3 months after the estradiol implants. We found that pituitary spheres from fetal alcohol exposed rat, express progenitor mesenchymal cell markers such as (OCT4, SOX2, KLF4, NANOG, CD133, Nestin and CD34). The gene ontology (GO) analyses of RNA-seq data of pituitary tumors from AF and AD reveal that most of the genes in AF animals are related to the mesenchymal characterization. We found that the Wnt5a and Wnt5b ligands, along with their cognate receptor Fzd, are generally overexpressed in pituitary tumor tissue of alcohol-exposed animals as compared to those in control animals. In addition, we found increased nuclear β-catenin (likely due to loss of E-cadherin), transcriptional activity of T cell factor (TCF), and several other growth factors including TGFβ, EGF, and FGF in the AF rat pituitaries. These data provide evidence for aggressive prolactinoma development due to overexpression of EMT factors involving Wnt/Frizzled signaling in the pituitary after estrogen

treatment in fetal alcohol exposed female rats. However, further studies need to be done to evaluate whether fetal alcohol exposure directly upregulates EMT pathway through Wnt/Frizzled signaling to increase aggressiveness in the pituitary gland. (This work is supported by a National Institute of Health grant R01 AA11591).

Disclosures: **S. Jabbar:** None. **W. Belden:** None. **J. Park:** None. **D.K. Sarkar:** None.

Poster

682. Nicotinic Acetylcholine Receptors: Structure and Regulation

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 682.01/F36

Topic: B.02. Ligand-Gated Ion Channels

Support: University of the Sciences start up

Title: Desformylflustrabromine, a positive allosteric modulator of the α4β2 nicotinic receptor, acts as an inhibitor of other heteromeric nicotinic receptor subtypes

Authors: *L. DECRISTOFANO, S. KHATRI, D. TORRES, M. SCHULTE;
Pharmaceut. Sci., Univ. of the Sci. in Philadelphia, Philadelphia, PA

Abstract: Nicotinic acetylcholine receptors are located throughout the central and peripheral nervous systems and have been implicated in many disorders including autism, depression, ADHD, and nicotine dependence. Members of the cys-loop family, nAChRs are composed of five subunits which can be assembled in a wide array of combinations that include α2-α9 and β2-β4 subunits. The α3β4 subtype of nAChR is found in the habenula and the interpeduncular nucleus where it may play a role in addiction pathways. Desformylflustrabromine (dFBr) is an established positive allosteric modulator of α4β2 nicotinic receptors and an inhibitor of α7 receptors; however, its effect on other nicotinic receptor subtypes has not been previously determined.
We examined the selectivity of dFBr on three heteromeric nicotinic receptors, α3β4, α4β4, and α3β2. Receptors were expressed in Xenopus laevis oocytes by micro injection of mRNA (300ng/µl) corresponding to the appropriate receptor subunits in α to β ratios of 1:1, 1:9, and 9:1. The effect of dFBr on each subtype was evaluated using two-electrode voltage clamp electrophysiology. Oocytes expressing nAChRs were exposed to dFbr alone to determine if it was capable of activating the receptors. Ach and dFBr were also co-applied to determine if dFBr was capable of inhibiting or modulating Ach induced activity.
Previous studies suggest that α4β2 assembles in both low sensitivity and high sensitivity isoforms. Ach dose response curves obtained from analysis of these receptors at different α/β

injection ratios supports this hypothesis. Data from these experiments also demonstrate that dFBr is an inhibitor of Ach induced responses on all three receptor subtypes. No positive allosteric modulation was observed, supporting the high selectivity of dFBr's potentiation at the α4β2 subtype. Based on these findings, we determined that both the α and β subunits contribute to dFBr's effects on different receptor subtypes.

Disclosures: L. Decristofano: None. **S. Khatri:** None. **D. Torres:** None. **M. Schulte:** None.

Poster

682. Nicotinic Acetylcholine Receptors: Structure and Regulation

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 682.02/F37

Topic: B.02. Ligand-Gated Ion Channels

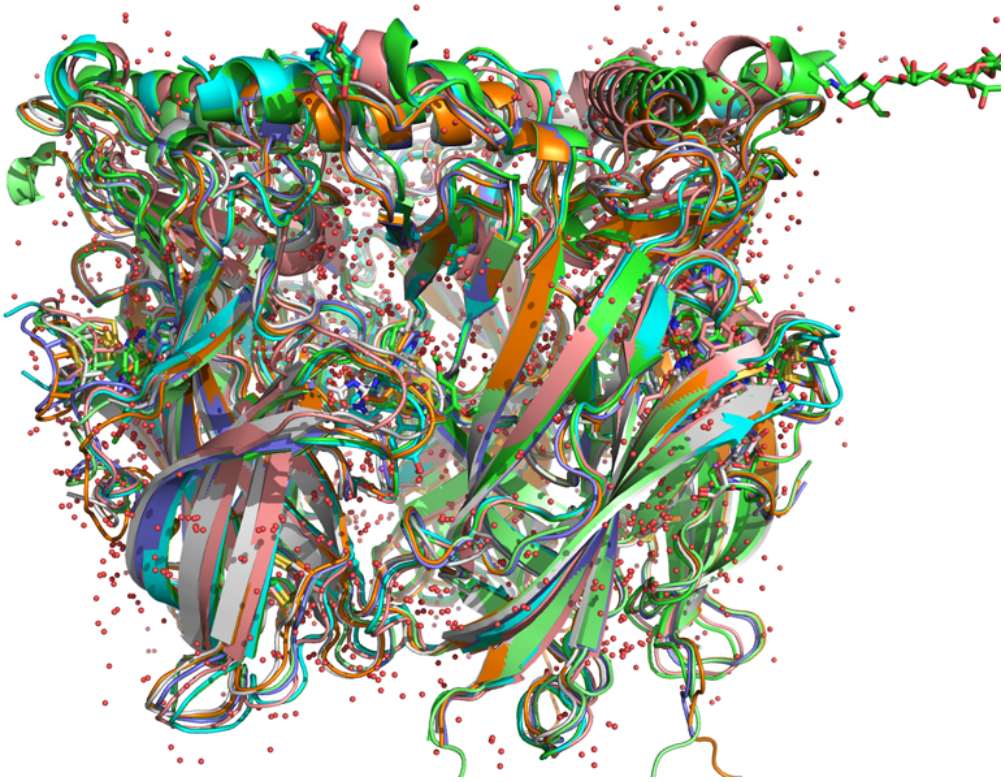
Support: ISU Seed Grant

ALSAM Foundation

Title: Engineered acetylcholine binding proteins for structure-guided drug design

Authors: *J. BOBANGO, S. WENSEL, I. TALLEY, T. T. TALLEY;
Dept. of Biomed. and Pharmaceut. Sci., Idaho State Univ., Meridian, ID

Abstract: Nicotinic acetylcholine receptors (nAChRs) are widely distributed throughout the CNS and periphery where they have crucial roles in physiological processes including learning, memory, and cognition. Consequently, nAChRs are potential targets for treating a variety of neurodegenerative disorders. Additionally, a number of natural products have evolved over time that target nAChRs and function for either protection or predation. Moreover, the nAChRs are the biological target of the fastest growing class of insecticides worldwide—the neonicotinoids. These pivotal roles necessitate a detailed understanding of the molecular determinates of ligand recognition and specificity for the nAChRs. The acetylcholine binding proteins (AChBPs) have been established as structural surrogates for the extracellular ligand binding domain of nAChRs and have contributed a great deal of information on the native receptor. Despite the utility of the AChBPs, they possess a distinct pharmacology to that of humans and other species of interest. To address this issue we have generated series of engineered AChBPs to evaluate potency and specificity of known and novel compounds. Furthermore, we have utilized X-ray crystallography for the direct observation of binding interactions at atomic-level resolution. Together, this allows us to contrast pertinent pharmacological information for structure-guided drug design.



Disclosures: **J. Bobango:** None. **S. Wensel:** None. **I. Talley:** None. **T.T. Talley:** None.

Poster

682. Nicotinic Acetylcholine Receptors: Structure and Regulation

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 682.03/F38

Topic: B.02. Ligand-Gated Ion Channels

Support: Barrow Neurological Foundation

Title: Chipping away at regulation of nicotinic acetylcholine receptor expression with NACHO

Authors: ***R. J. LUKAS**, J. B. EATON, L. LUCERO, P. WHITEAKER, A. A. GEORGE; Barrow Neurol Inst., Phoenix, AZ

Abstract: Nicotinic acetylcholine receptors (nAChR) are expressed extensively in all compartments of the nervous system, in skeletal muscle, and in other organs or systems such as the immune system. In these locations, nAChR play critical roles in health and have been

implicated in many disease processes and medical conditions. nAChR exist as a family of ligand-gated ion channel subtypes assembled as pentamers of different combinations of subunits encoded by a family of sixteen different genes. The law of identity (A is A) dictates that a native nAChR subtype and a heterologously expressed nAChR subtype of the same subunit composition and arrangement must have the same elemental properties. However, the local milieu can differentially influence observable properties of a given nAChR subtype. It sometimes has been a struggle to heterologously express some nAChR subtypes to facilitate comparing their properties to those of native nAChR. Examples are the failures using COS, CHO, HEK or other cells lines as hosts compared to our success in heterologously and stably expressing functional, human α7-nAChR (i.e., composed of α7 subunits) in the human epithelial cell line, SH-EP1. A multi-transmembrane domain protein has been identified recently ["NACHO" - Gu, Bredt and others, Neuron (2016) 89:1-8] that resides in the endoplasmic reticulum (ER) and promotes α7-nAChR expression, indicating importance of cell type-specific mediation of nAChR functional levels. NACHO was initially discovered, by high throughput screening, in a rare, human HEK cell variant capable of expressing functional α7-nAChR. We now demonstrate abundant expression of NACHO as mRNA in SH-EP1 cells, helping to explain the ability of those cells to stably express α7-nAChR. We also have confirmed potent facilitation of α7-nAChR functional expression in *Xenopus* oocytes also expressing NACHO, and at levels much higher than those in the presence of another chaperone, Ric-3. This occurs even for concatenated α7-nAChR, suggesting that NACHO facilitates exit of assembled receptors from the ER, although it may also promote assembly from loose subunits. Additional studies concern effects of NACHO on functional expression of other nAChR subtypes and, reciprocally, influences of nAChR functional modulation on NACHO expression. At a minimum, and as opposed to use of allosteric modulation, which alone could affect receptor properties, NACHO is a very useful research tool to "naturally" elevate functional expression of nAChR.

Disclosures: **R.J. Lukas:** None. **J.B. Eaton:** None. **L. Lucero:** None. **P. Whiteaker:** None. **A.A. George:** None.

Poster

682. Nicotinic Acetylcholine Receptors: Structure and Regulation

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 682.04/F39

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant NS59910

Title: Optogenetic activation of striatal cholinergic interneurons and D2 receptor-expressing GABAergic medium spiny neurons regulates tardive dyskinesia.

Authors: *T. BORDIA, D. ZHANG, X. A. PEREZ, M. QUIK;
Ctr. for Hlth. Sci., SRI Intl., Menlo Park, CA

Abstract: Tardive dyskinesia (TD) is a potentially irreversible drug-induced movement disorder that arises as a side effect of antipsychotics. These drugs are the mainstay of treatment for schizophrenia and bipolar disorder. They are also increasingly prescribed for major depressive disorder, autism, attention deficit hyperactivity disorder, obsessive compulsive disorder and post-traumatic stress. There is thus a need for therapies to reduce TD. Previous work showed that nicotine administration decreased haloperidol-induced vacuous chewing movements (VCMs) in a rat TD model. The present experiments demonstrate that nicotine (300 µg/ml in drinking water) also reduced antipsychotic-induced VCMs (~50%) in mice whether the antipsychotic haloperidol was given via constant infusion (subcutaneous pellet 3 mg/kg/d) or injection (1 mg/kg twice daily). To elucidate the pathways and mechanisms that underlie TD, we used optogenetics with a focus on the striatum because of the close link of this region to TD. Optical stimulation of striatal cholinergic interneurons using choline acetyltransferase (ChAT)-Cre mice expressing ChR2-eYFP decreased haloperidol-induced VCMs (~50%), with no effect in control-eYFP mice. Activation of striatal D2 medium spiny neurons using ChR2-eYFP expressing Adora2a-Cre mice also diminished antipsychotic-induced VCMs, again with no effect of stimulation in control-eYFP mice. The stimulation-induced declines in VCMs occurred via nicotinic acetylcholine receptors in both ChAT-Cre and Adora2a-Cre mice. Molecular studies indicate c-Fos activation plays a role. Overall, this work indicates that striatal cholinergic interneurons and GABAergic neurons play an important role in TD. Moreover, these studies suggest that nAChR drugs may be useful for reducing antipsychotic-induced TD.

Disclosures: **T. Bordia:** None. **D. Zhang:** None. **X.A. Perez:** None. **M. Quik:** None.

Poster

682. Nicotinic Acetylcholine Receptors: Structure and Regulation

Location: Halls B-H

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Program#/Poster#: 682.05/F40

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH R01ES03299

NIH R25NS065777

Title: Role of the homo and heteromeric nicotinic acetylcholine receptor in mediating cytotoxicity after delayed methylmercury exposure in PC12 cells

Authors: *M. RIOS CABANILLAS[1], W. D. ATCHISON[2];
[1]Pharmacol. and Toxicology, [2]Michigan State Univ., East Lansing, MI

Abstract: Acute exposure to methylmercury (MeHg) disrupts internal calcium (Ca^{2+}) homeostasis and causes subsequent cell death. The pathways involved during the MeHg-induced increase in internal Ca^{2+} concentration ($[Ca^{2+}]_i$) and cytotoxicity are still not completely identified. Studies show that nicotinic cholinergic receptors (nAChRs) appear to contribute to MeHg-induced cytotoxicity in differentiated sympathetic neuron-like PC12 (dPC12) cells. Exposure to the nonspecific nAChR antagonist, mecamyllamine (MEC), increases cell viability after acute MeHg exposure. As different nAChR subtypes act in cell-type specific manner, and have differential Ca^{2+} affinities, we compared the role of the highly Ca^{2+}-permeable homomeric α7 subtype nAChR with other heteromeric nAChRs, to the contribution of MeHg-induced cytotoxicity. Cell viability was assayed using the calcein AM and ethidium homodimer-1 (Ethd-1). Calcein AM stains cytoplasm of live cells while Ethd-1 stains DNA of dead cells. dPC12 cells were exposed to 1, 2 or 5 μM MeHg during 1 hr in the absence or presence of the specific (α7) or nonspecific blockers: MLA (5 μM) or MEC (5 μM), respectively. Delayed viability measurements after MeHg exposure during 1 hr significantly decreases dPC12 cells viability in a concentration-dependent manner. At 2 μM MeHg, presence of either MEC or MLA treatments alone significantly increases viability by 37% and 32%, respectively. Furthermore, combination of MEC + MLA together significantly increases the protection provided by either antagonist alone by 16%. At 5 μM MeHg only MEC treatment significantly increased dPC12 cell viability by 39% from MeHg treatment alone, and the combination of MEC + MLA by 22.44%. At high MeHg concentration MLA treatment alone significantly increases cell viability by 14.28% only from the MEC group (21%) but not MeHg alone. Data suggest that the α7-subtype nAChR is not the only receptor contributing to MeHg-induced cell death but that other nAChR subtypes may also play a role. Supported by NIH grant R01ES03299 & R25NS065777.

Disclosures: M. Rios Cabanillas: None. **W.D. Atchison:** None.

Poster

682. Nicotinic Acetylcholine Receptors: Structure and Regulation

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 682.06/F41

Topic: B.02. Ligand-Gated Ion Channels

Title: Cell type-dependent TMEM35 expression and its effects on surface α7 nicotinic acetylcholine receptors

Authors: *R. H. LORING, B. GARG, H. LIN, A. REZVAYA, B. TILAK;
Pharmaceut. Sci., Northeastern Univ., Boston, MA

Abstract: Surface α7 nicotinic acetylcholine receptor (nAChR) expression depends on the cell type, possibly due to differences in endoplasmic reticulum chaperone proteins that allow folding, assembly and transport to the cell membrane. RIC3 (Resistance to inhibitors of cholinesterase 3) is a known chaperone for α7 nAChR. However, we previously found no significant RIC3 protein expression in GH4C1, a cell line derived from rat pituitary, even though these cells readily produce surface α7 receptors measured by α-bungarotoxin binding when transfected with α7 nAChR plasmid. Further, surface expression in GH4C1 cells is not changed by RIC3 shRNAs that block surface α7 expression in other cell lines that require RIC3 transfection (Koperniak et al, *J. Neurochem.* **124:** 300, 2013). Together, these data suggested that GH4C1 cells have a different α7 receptor chaperone than RIC3. Recently (3/2/2016), Gu et al. (*Neuron* **89:** 1, 2016) reported that Transmembrane Protein 35 (TMEM35, also known as NACHO) acts as another chaperone in HEK293 cells when expressed alone with α7 and also synergistically enhances RIC3 effects. We investigated how widely TMEM35 is expressed in cell lines that have little or no endogenous α7 nAChRs and compared those cells lines for surface expression of transfected α7. GH4C1 and GH3 cells readily express surface α7 when transfected, while SH-SY5Y has minor endogenous α7 expression, and SH-EP1, HEK293, RAW264.7 and H9C2 cells showed no expression when transfected. Western blots showed a rank order for TMEM35 protein expression of GH3 ≥ GH4C1 >> SH-SY5Y, with no detectable expression in other cells. These data suggest a correlation between the level of endogenous TMEM35 protein and the ability of unmodified cell lines to express surface α7 when transfected. However, preliminary binding experiments suggest that TMEM35 tagged with C terminal GFP does not significantly enhance α7 surface expression in HEK cells co-transfected with RIC3 and α7, while TMEM35 tagged with C terminal Myc-DDK antigens significantly decreases surface expression. In contrast, TMEM35-GFP permits significant surface α7 expression in H9C2 cells when transfected with α7 in the absence of RIC3. If confirmed with proper controls, these data may suggest an interaction between RIC3 and the C-terminal portion of TMEM35 that helps regulate surface α7 expression in different cell types.

Disclosures: R.H. Loring: None. **B. Garg:** None. **H. Lin:** None. **A. Rezvaya:** None. **B. Tilak:** None.

Poster

682. Nicotinic Acetylcholine Receptors: Structure and Regulation

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 682.07/F42

Topic: B.02. Ligand-Gated Ion Channels

Title: G protein-coupled receptor (GPCR) signaling underlies the nicotine-induced up regulation of alpha 7 (α7) nicotine acetylcholine receptors (nAChRs) expressed in Xenopus oocytes

Authors: *J. PANCHAL[1,2], K. DEBOEUF[2], M. ISLAM[2], J. FARLEY[2];
[2]Neurosci., [1]Indiana Univ., Bloomington, IN

Abstract: α7 nAChRs are widely distributed throughout the nervous system, playing important roles in learning & memory, and are also implicated in a variety of disease and neurodegenerative processes including schizophrenia, Alzheimer's and Parkinson's disease, inflammation, pain, cancer, and nicotine addiction (Thomsen et al 2010, Bencherif et al 2011, Changeux et al 2012). A variety of compounds (agonists, antagonists, PAMs) produce functional and/or numerical upregulation of α7 Rs in different cells, implicating multiple signaling pathways and mechanisms. Prolonged nicotine exposure can also upregulate α7 nAChRs, which may contribute to nicotine dependence and addiction (Govind et al 2012, Brunzell et al 2014). Previously, we found ~ 2-fold functional and numerical upregulation of murine α7 nAChRs in *Xenopus* oocytes following 12 hr of 100 μM nicotine and extensive washout. We found that nicotine-upregulation was dependent upon intracellular Ca^{2+}, being abolished by BAPTA-AM, and involved several Ca^{2+}-dependent enzymes (e.g., PP2B, PKC). But upregulation was independent of Ca^{2+} influx, being unaffected by removal of extracellular Ca^{2+}. Similar to another pentameric, Cys-loop LGIC, glycine receptor 1 (GlyR1), the α7 nAChR contains a conserved G protein-binding cluster (GPBC) in the M3-M4 loop. G-protein signaling by α7 Rs has been shown in both neurons and PC12 cells (Kabbani et al 2013). Here, we show that GPCR signaling mediates nicotine-upregulation of α7 nAChR. Mutation of α7 nAChR GPBC (RMKR to AAAA; denoted as α7 344-347A) blocks interaction of Gαq and Gβγ with the GPBC without affecting receptor expression, peak current amplitude or kinetics. However, nicotine-upregulation of α7 nAChR did not occur for α7 344-347A Rs. Conversely, 12 hr exposure to the cell-permeable, competitive antagonist Methyllycaconitine (MLA), produced ~ 2-fold upregulation of both wt and mutant (α7 344-347A) α7 Rs; and upregulation was unaffected by BAPTA-AM. Our results suggest that α7 nAChRs may function as both metabotropic and ionotropic receptors, and that GPCR-signaling is critical for nicotine-upregulation.

Disclosures: **J. Panchal:** None. **K. DeBoeuf:** None. **M. Islam:** None. **J. Farley:** None.

Poster

682. Nicotinic Acetylcholine Receptors: Structure and Regulation

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 682.08/F43

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant NS-047332

NIH Grant NS-082615

Title: AChRs are essential for the targeting of rapsyn to the postsynaptic membrane of NMJs in living mice

Authors: *P.-J. CHEN, I. MARTINEZ, M. AITTALEB, M. AKAABOUNE;
Mol. Cell. and Developmental Biol., Univ. of Michigan, Ann Arbor, MI

Abstract: Rapsyn, a 43 kDa scaffold protein, is required for the clustering of acetylcholine receptors (AChRs) at synaptic sites between mammalian motor neurons and muscle cells. However, the mechanism by which rapsyn is inserted and retained at postsynaptic sites at the neuromuscular junction (NMJ) *in vivo* remains largely unknown. We found that neither the N-terminal myristoylation nor the cysteine-rich RING H2 domain of rapsyn is required for its stable association with the postsynaptic membrane of NMJs. When N-myristoylation-defective rapsyn-EGFP mutant (G2A) and RING-H2 domain truncated rapsyn-EGFP were electroporated into sternomastoid muscles, a strong rapsyn fluorescent signal was selectively observed at synapses, similar to wildtype rapsyn-EGFP. The targeting of rapsyn-EGFP (wildtype and mutants) is independent of synaptic activity as they were inserted at denervated NMJs. However, when the coiled-coil domain (the AChR binding domain of rapsyn) is deleted, rapsyn fails to associate with AChRs at NMJs of living mice. In cultured myoblasts (in which AChRs are absent), myristoylated wildtype rapsyn mostly localizes to lysosomes, and is not associated with plasma membrane. However, in the presence of AChR subunits, rapsyn molecules were targeted to cell surface and formed aggregates with AChRs. The targeting of AChRs to the cell membrane, in contrast, does not require rapsyn as expressed AChRs are visible on cells membrane of rapsyn deficient myoblasts. These results provide evidence for an active role of AChRs in the targeting of rapsyn to the NMJ *in vivo*.

Disclosures: **P. Chen:** None. **I. Martinez:** None. **M. Aittaleb:** None. **M. Akaaboune:** None.

Poster

682. Nicotinic Acetylcholine Receptors: Structure and Regulation

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 682.09/F44

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH P20GM103466

Title: New nicotinic acetylcholine receptor ligands using privileged scaffolds and the hybrid approach

Authors: *D. GUNDISCH, M. J. CALIBUSO-SALAZAR, I. TOMASSOLI;
Col. of Pharm. UHH, Hilo, HI

Abstract: Recently, we designed and synthesized “twin” compounds targeting nicotinic acetylcholine receptors (nAChRs) (Bioorg Med Chem, 2015, 23(15):4375-4389), and a new lead compound with high subtype selectivity for beta2-containing nAChRs has been obtained from this approach. Using a similar design approach we prepared small in silico ADME guided compound libraries based on diaza(bi)cyclic scaffolds and various established central nervous system (CNS) drugs forming so-called hybrid compounds. Diaza(bi)cyclic motifs are known to be privileged scaffolds for the interaction with various related biological targets including nicotinic acetylcholine receptors (nAChRs). This project focuses on the development of small libraries of compounds interacting with nAChRs and other biological targets which could be relevant for an improved treatment of CNS disorders like e.g. major depression, substance use disorder, and Alzheimer’s disease. For structure affinity relationship evaluation on nAChRs, compounds were tested in radioligand binding assays with [3H]epibatidine, [3H]methyllycaconitine, [3H or 125I]alpha-bungarotoxin, [3H]NS14492 and [3H]NS10743 using membrane fractions of rat brains, pig brains, pig adrenals, and Torpedo californica electroplax. A broad spectrum of affinities (Ki values: < 10 nM to > 10,000 nM) and subtype selectivity for nAChRs provided important hints for further “designing in”, “balancing”, and “designing out” strategies to obtain new active compounds for future lead candidates.

Disclosures: D. Gundisch: None. **M.J. Calibuso-Salazar:** None. **I. Tomassoli:** None.

Poster

682. Nicotinic Acetylcholine Receptors: Structure and Regulation

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 682.10/F45

Topic: B.02. Ligand-Gated Ion Channels

Title: Extracellular domain α4β2 nicotinic acetylcholine receptors form with specific homomeric or swapped M1 transmembrane domains

Authors: *G. B. WELLS[1], A. M. PERSON[2];
[1]Mol. & Cell. Med., [2]Mol. and Cell. Med., Texas A&M Univ. Hlth. Sci. Ctr., College Station, TX

Abstract: Background: Extracellular domain (ECD) receptors derived from α7, α4, α3, β2, and β4 nicotinic receptor subunits truncated after the first transmembrane domain (M1) have functional and structural similarity to full length nicotinic acetylcholine receptors (nAChRs). Their smaller size and reduced transmembrane sequence might have advantages for structural studies by crystallography and NMR and for understanding specific structural and functional roles of the ECD and transmembrane domains in nAChRs and other Cys-loop receptors. Chimeras of M1 suggest that M1 requires specific sequences to fulfill this role. For example, M1 from α7 or 5HT3 is an inefficient replacement for native M1 sequences in α4β2 ECD nAChRs. Important properties of M1 sequences for expressing ECD nAChRs, however, are uncertain.
Objective: To determine the efficiency of expressing α4β2 ECD nAChRs with swapped and with homomeric M1 sequences from α4 and β2. Combined with results from chimeras from M1 domains of α7 and serotonin 5HT3A subunits, these data help identify important features in M1 for expressing α4β2 ECD nAChRs and other Cys-loop subunits.
Methods: Human α4 and β2 cDNAs were truncated after M1 (α4M1 and β2M1). M1 domains were swapped between α4M1 and β2M1 subunits with site directed mutagenesis. α4β2 ECD nAChRs were expressed in *Xenopus* oocytes either with swapped M1 domains or with homomeric M1 domains. In addition, the residue juxtaposed N-terminal to M1 also was swapped (native **L** in α4M1 or native **K** in β2M1), either in isolation or along with M1. Immunoblotting and yield of [^{3}H]epibatidine binding sites assessed effects on expression.
Results: α4β2 ECD nAChRs with homomeric M1 from α4 or from β2 expressed at levels comparable to native α4β2 ECD nAChRs. α4β2 ECD nAChRs with swapped M1 domains expressed at levels comparable to native α4β2 ECD nAChRs. Placing leucine ahead of M1 in β2M1 diminished relative expression of the α4β2 ECD nAChR with this homomeric leucine site to about 5%. Placing M1 from α4 into β2M1 leading to a M1 homomer for α4 and β2 subunits did not rescue expression with this leucine. In contrast, placing lysine ahead of M1 in both α4M1 and β2M1 or swapping leucine and lysine between α4M1 and β2M1 led to good expression of α4β2 ECD nAChRs.

Conclusions: α4β2 ECD nAChRs express well with homomeric M1 from either α4 or β2 but not M1 from α7 or 5HT3A. The residue N-terminal to M1 is important for expressing α4β2 ECD nAChRs and appears to require or tolerate a positive change in either α4M1 or β2M1. Presence of a leucine in both subunits is not tolerated. These results help refine the concept of an optimally designed M1 sequence for ECD Cys-loop receptors.

Disclosures: G.B. Wells: 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

682. Nicotinic Acetylcholine Receptors: Structure and Regulation

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 682.11/F46

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH (NINDS) Grant: NS077114

Title: Studying the structure, function and interactions of the intracellular domain of Cys-loop receptors

Authors: A. PANDHARE[1], E. PIRAYESH[1], P. N. GROZDANOV[2], *M. JANSEN[1];
[1]Cell Physiol. Mol. Biophys., [2]Cell Biol. Biochem., TTUHSC, Lubbock, TX

Abstract: The intracellular domain (ICD) of metazoan Cys-loop receptor superfamily members, also known as pentameric ligand-gated ion channels (pLGICs), has been the least explored domain. Structure/function studies over decades, including recent crystal structures for mammalian pLGICs, have vastly enriched our understanding of the other two, extracellular (ECD) and transmembrane (TMD), domains. The variable lengths and amino acid sequences confer the most diversity to the ICD contrary to the highly-conserved ECD and TMD. This unique feature of being a starkly diverse domain poses its own challenges by necessitating to develop an 'individualized' approach to study structure, function and interactions of ICDs. In addition, the *a priori* assumption of the ICD being largely disordered or disorganized structurally has been prevailing in the field for decades. In our recent study, we made the surprising discovery that the 5-HT_{3A} ICD alone assembles into stable pentamers in solution (Pandhare, A. et al. *Sci. Rep.* **6**, 23921(2016)). The finding suggests a novel role for the ICD in receptor oligomerization, and significantly adds to its currently known repertoire of functions. To further expand our study across the Cys-loop receptor superfamily and, importantly, to address the huge knowledge gap pertaining to structure, function and interactions of each unique ICD, we have engineered individual chimeras comprising of ICDs of prominent pLGICs subunits. Current

studies aim at elucidating the molecular determinants of pentameric oligomerization as well as measuring affinities of self-association, investigating protein-protein interactions with non-homologous proteins, and in addition pursuing structural studies at atomic resolution and importantly determining functional contributions of the ICD.

Disclosures: A. Pandhare: None. **E. Pirayesh:** None. **P.N. Grozdanov:** None. **M. Jansen:** None.

Poster

682. Nicotinic Acetylcholine Receptors: Structure and Regulation

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 682.12/F47

Topic: B.02. Ligand-Gated Ion Channels

Title: Brain α7 nicotinic acetylcholine receptor assembly requires nacho

Authors: *S. GU;
Neurosci., Janssen Res. & Development,, San Diego, CA

Abstract: Neuronal nicotinic acetylcholine receptors (nAChRs) are ligand-gated ion channels that participate in diverse physiological responses such as pain processing and cognitive functions. Alpha7 nACh receptors are homo-pentameric, and they do not oligomerize or functionally express in most all non-neuronal cell lines. Up-regulation of nAChR in brain represents a therapeutic goal and likely contributes to the pharmacology of cholinergic medicines in Alzheimer's disease and schizophrenia. Whereas some neurotransmitter receptors have auxiliary subunits that control receptor trafficking and channel gating, no essential assembly proteins have previously been identified for a mammalian neurotransmitter receptor. Using a genomic cDNA screening strategy, we identified a unique, phylogenetically conserved, four-pass transmembrane protein, NACHO, which is essential for functional α7 receptors in transfected cells. NACHO selectively promotes function of α7 and α4β2 nAChRs and does not influence a related 5-HT_3 receptor. NACHO is a neuronal, endoplasmic reticulum protein that enhances both assembly and surface trafficking of α7 receptors. Genetic disruption of NACHO completely disrupts assembly of α7 pentamers and abolishes functional α7 receptors. This work identifies NACHO as an essential, client-specific chaperone for nAChRs and has implications for physiology and disease associated with these widely-distributed neurotransmitter receptors.

Disclosures: S. Gu: None.

Poster

682. Nicotinic Acetylcholine Receptors: Structure and Regulation

Location: Halls B-H

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Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant DA037492

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HHMI Gilliam fellowship

McKnight Scholar Award

Klingenstein-Simons Fellowship Award

Title: Toward the structure and molecular mechanisms of the human $\alpha_4\beta_2$ nicotinic acetylcholine receptor

Authors: *L. C. MORALES, C. NOVIELLO, R. HIBBS;
Neurosci., UT Southwestern Med. Ctr., Dallas, TX

Abstract: Nicotinic acetylcholine receptors mediate fast chemical neurotransmission in the central and peripheral nervous systems. The $\alpha_4\beta_2$ subtype is the most abundant nicotinic acetylcholine receptor in the human brain. The long term goals of this research project is to obtain a high-resolution structure of the $\alpha_4\beta_2$ nicotinic receptor to probe the allosteric gating, ion selectivity and ligand recognition mechanisms. Here we report biochemical and biophysical studies of a modified construct that shows an improvement in expression, homogeneity and pentameric monodispersity. We have successfully optimized our expression and purification methods to obtain milligram quantities of homogenous receptor. In addition, we have performed patch-clamp experiments and binding assays demonstrating that the modified version of the receptor is functional. To promote crystallization we have screened ligands, detergents, lipids and additives against our best construct to obtain high quality crystals. A high-resolution crystal structure will provide important insights into the molecular mechanisms of activation and selectivity of this family of ligand-gated channels.

Disclosures: L.C. Morales: None. **C. Noviello:** None. **R. Hibbs:** None.

Poster

682. Nicotinic Acetylcholine Receptors: Structure and Regulation

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 682.14/F49

Topic: B.02. Ligand-Gated Ion Channels

Title: Evaluation of methyllycaconitine (MLA) analogues on α_7 and $\alpha_4\beta_2$ nAChR subtypes.

Authors: *T. Z. QUDAH[1], R. GALLAGHER[2], N. ABSALOM[1], T. BALLE[1], M. MCLEOD[2], M. CHEBIB[1];
[1]Fac. of Pharm., Univ. of Sydney, Camperdown, Australia; [2]The Australian Natl. Univ., ACT, Australia

Abstract: Nicotinic acetylcholine receptors (nAChRs) are pentameric ligand-gated ion channels where the α7 and α4β2 subtypes are the most predominant in the brain. The α4β2 nAChR is known to exist in two functional isoforms with different ACh-activation properties, namely the high sensitivity (HS) $(\alpha4)_2(\beta2)_3$ and low sensitivity (LS) $(\alpha4)_3(\beta2)_2$ receptor[1]. We and others have demonstrated that a large number of pharmacological differences between the two receptors can be attributed to the presence of an additional agonist binding site at the α4-α4 interface on $(\alpha4)_3(\beta2)_2$ receptor. MLA is natural toxic potent antagonist that competes with ACh at the same binding site. MLA is selective for α7 receptors with approximately 1000-fold greater potency than at α4β2. Identifying ligands that are selective for specific nAChR subtypes through their affinity for different binding interfaces may have significant therapeutic potential and contribute to the understanding of the physiological roles of these subtypes *in vivo*.
Previously, we have highlighted the importance of the N-succinemidoanthranilate moiety for binding at the α7 subtype[2].In this study, we propose that the AE succinimide structure of MLA has higher selectivity at the α4-α4 interface in $(\alpha4)_3(\beta2)_2$ over the α7-α7 interface. We have synthesised different MLA analogues and evaluated them by co-applying 10 μM of each compound with 1 mM ACh for $(\alpha4)_3(\beta2)_2$ and α7 and 100 μM $(\alpha4)_2(\beta2)_3$ on recombinant human α_7 and two different stochiometries of α4β2 expressed in *Xenopus* oocytes using the two-electrode voltage clamp technique.To date, we have identified three analogues (BA09, BA11 and BA12) that are able to inhibit the current at $(\alpha4)_3(\beta2)_2$ by 80%, 82% and 70% with no antagonism at the α7 subtype, when compared to 100% inhibition by 10 μM MLA at both subtypes. The same analogues on $(\alpha4)_2(\beta2)_3$, only inhibited the evoked ACh current by 35%, 30% and 33% respectively. Based on this result, we are able to design analogues that distinguish between α4β2 and α7 receptors, however further refinement of the chemical structures are required to achieve greater selectivity between the two α4β2 stochiometries.
1) Harpsøe K *et al* (2011). J Neurosci,31,10759-66.
2) Quek GX *et al* (2010). ACS Chem Neurosci. 12,796-809.

Disclosures: **T.Z. Qudah:** None. **R. Gallagher:** None. **N. Absalom:** None. **T. Balle:** None. **M. McLeod:** None. **M. Chebib:** None.

Poster

682. Nicotinic Acetylcholine Receptors: Structure and Regulation

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 682.15/F50

Topic: B.02. Ligand-Gated Ion Channels

Support: John A. Lamberton research fellowship

Title: Differential pharmacology of nicotinic receptor mutations that cause Autosomal Dominant Frontal Lobe Epilepsy at two different stoichiometries

Authors: ***D. INDURTHI**[1], T. QUDAH[2], T. BALLE[1], N. L. ABSALOM[1], M. CHEBIB[1]; [1]The Univ. of Sydney, Camperdown, Australia; [2]Univ. of Sydney, Camperdown, Australia

Abstract: Introduction: Epilepsy is central nervous system (CNS) disorder characterized by recurring seizures, affecting in 1-2% of the world population. Several mutations have been identified in the α4, β2 and α2 subunits of neuronal acetylcholine receptors (nAChRs) that cause nocturnal frontal lobe epilepsy (NFLE) that is autosomal dominant in nature called ADNFLE. The α4β2 nAChR is the most abundant heteromeric nAChR in the brain, which is known to exist in two functional isoforms $(\alpha4)_2(\beta2)_3$ and $(\alpha4)_3(\beta2)_2$. We and others have extensively characterized the two isoforms and attributed pharmacological differences, to the presence of additional agonist binding site at α4-α4 interface on $(\alpha4)_3(\beta2)_2$ receptor. Very little is known on the molecular and pathophysiological mechanisms on how the ADNFLE mutations alter the native functioning of the two isoforms α4β2. **Aim:** To understand the functional changes caused by the α4 (T293I) and β2 (V287M & V287L) ADNFLE mutations that are located around extracellular domain that is in contact with the trasmembrane domain (TM2-TM3 loop). **Results and conclusion:** Using two-electrode electrophysiology and determining open probability estimates, we show that these mutants increase the efficacy of activation by the native ligand, acetylcholine (ACh), by nearly two-fold. We then studied the pharmacology of mutant receptors with several clinically-relevant ligands, including the smoking-cessation drug, varenicline, the anti-epileptic drug, carbamazepine and a selective agonist at α4β2 receptors, Sazatidine-A. Surprisingly, we observe that mutations on α and β subunits, present at the same location on the receptor have different pharmacological profiles when expressed in different stoichiometries. The increased efficacy most likely leads to the increased electrical activity in the brain that causes seizures. Understanding the pharmacology of these mutant receptors has a therapeutic potential to find a drug target to cure the disease. [1]Authors would like to acknowledge the

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Disclosures: **D. Indurthi:** None. **T. Qudah:** None. **T. Balle:** None. **N.L. Absalom:** None. **M. Chebib:** None.

Poster

682. Nicotinic Acetylcholine Receptors: Structure and Regulation

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 682.16/F51

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant R21 DA027070

NIH Grant R21 DA036059S

Title: α7 and α7β2 nicotinic receptor (nAChR) subtypes are differentially modulated by the endogenous membrane-associated prototoxins, lynx1 and lynx2

Authors: *A. A. GEORGE[1], J. B. EATON[1], R. J. LUKAS[1], J. M. MIWA[2], P. WHITEAKER[1]; [1]Neurobio., Barrow Neurolog. Inst., Phoenix, AZ; [2]Biol. Sci., Lehigh Univ., Bethlemhem, PA

Abstract: Nicotinic acetylcholine receptors (nAChR) exist as pentameric complexes of homologous, but genetically distinct subunits. The functional expression of nAChR mediates aspects of synaptic transmission, and nAChR dysfunction can be linked to many neurodegenerative and psychiatric diseases. The α4β2 and α7 nAChR subtypes are the most abundantly expressed subtypes in the mammalian central nervous system. α7 and α7β2 nAChR are predominantly expressed in the cortex, hippocampus, amygdala and basal ganglia. Moreover, these ligand-gated ion channels influence cellular excitability and homeostatic regulation in basal forebrain cholinergic neurons. While α7 and α7β2 nAChR mediate the cellular processes involved in many neurological processes, nAChR function can, in turn, be altered by endogenous neuropeptides. The Ly6 family of neuromodulators can influence the functional expression of many different nAChR subtypes including α7*, α4β2* and α3β4* nAChR (*indicating the presence of additional subunits). Two members of the Ly6 family, lynx1 and lynx2, have been shown to enhance desensitization and decrease sensitivity of nAChR to acetylcholine. However, the ability of lynx1 and lynx2 to interact with α7 and α7β2 nAChR is poorly understood. In the current line of investigation, we used a concatenated (i.e. linked) subunit approach to ensure consistent subunit incorporation while probing the functional modulation of α7 and α7β2 nAChR by lynx1 and lynx2. Using two-electrode voltage clamp (TEVC) electrophysiology, we

demonstrate that lynx1 attenuates macroscopic receptor peak currents for both α7 and α7β2 nAChR subtypes when expressed in *Xenopus* oocytes. Using a range of lynx1 doses, we demonstrate that significant functional blockade of both α7 and α7β2 subtypes can be achieved at a 1:1 nAChR:lynx RNA injection ratio (producing 90 ± 7.5% and 95 ± 11% reductions in maximal function, respectively). Furthermore, we demonstrate that coexpression with lynx1 fails to produce a significant shift in either α7 or α7β2 ACh concentration-response profiles. Co-expression of α7 or α7β2 nAChR with lynx2 also produced a similar reduction in macroscopic function, but the magnitude of the effect was reduced relative to that for lynx1. The current findings suggest that alterations in α7 and α7β2 nAChR function by lynx1 and/or lynx2 might be a novel and productive therapeutic strategy to mitigate cognitive decline associated with neurodegenerative disease. This work was supported by the Barrow Neurological Foundation Award (A.A.G) and by NIH grants R21 DA027070 and R21 DA036059S (P.W).

Disclosures: **A.A. George:** None. **J.B. Eaton:** None. **R.J. Lukas:** None. **J.M. Miwa:** None. **P. Whiteaker:** None.

Poster

682. Nicotinic Acetylcholine Receptors: Structure and Regulation

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 682.17/F52

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH-NIDA Grant DA036673

Title: Serine residues in the MIII-MIV intracellular loop of the α4 nAChR subunit control α4β2* nAChR expression in the neuronal surface

Authors: *C. A. ZAMBRANO[1], D. ESCOBAR[1], J. A. STITZEL[1,2];
[1]Inst. for Behavioral Genet., [2]Dept. of Integrative Physiol., Univ. of Colorado, Boulder, CO

Abstract: Chronic nicotine exposure produces an increase or upregulation of α4β2* nAChR expression in neurons. The mechanism(s) that produce α4β2* nAChR upregulation represents a pharmacological target to control its behavioral consequence. The aim of this work is to find a relationship between receptor structure and activity for the α4β2 nAChR that allow us to study mechanisms of nicotine-induced nAChR upregulation. The α4 subunit has an intracellular loop between MII-MIV transmembrane domains that contains serine residues previously described as potential phosphorylation substrates for protein kinase A and protein kinase C in heterologous expression systems. Our studies are focused on residues S336, S470 and S530. Adeno associated virus (AAV) containing the wild type mouse α4 nAChR cDNA or cDNAs for each of the three

serine to alanine point mutations S336A, S470A and S530A were produced. Primary neuronal cultures prepared from cerebral cortex, hippocampus and brainstem of α4 KO mouse embryos were infected with those vectors and chronically exposed with nicotine to measure α4β2* nAChR binding sites using [^{125}I]epibatidine. A triple HA tag was added at the C-terminal of the α4 subunit. Detection with HA antibodies revealed the formation of clusters of α4β2* nAChR that are expressed in the neuronal surface after 10 days of AAV infection. Following nicotine treatment, the α4 mutants S336A and S470A failed to show upregulation of surface receptors in hippocampal and brainstem neurons. This effect appears to be cell type specific as normal surface upregulation of the mutants as observed in cortical neurons. Data suggests that multiple mechanisms of upregulation exists for neurons obtained from different brain regions possibly due to a differential interaction with kinases or phosphatases that control surface expression of α4β2 nAChR. The mutations are also being study in vivo. AAVs containing the wild type and mutated α4 nAChR subunit cDNAs are being injected in the dorsal hippocampus of α4 KO mice, followed by chronic nicotine intravenous infusion (2 mg/kg/h). Preliminary data confirms α4-nAChR subunit re-expression in the dorsal hippocampus but also retrograde expression was observed in the entorhinal cortex. [^{125}I]epibatidine binding was detected after AAV infection confirming α4β2* nAChR expression. An increase in [^{125}I]epibatidine binding was observed in α4 wild type cDNA infected mice that received chronic nicotine infusion for 10 days compared with saline infused mice. We plan to use this model to correlate the effect of differential α4β2 nAChR upregulation with memory impairment after chronic nicotine withdrawal using the contextual fear conditioning test.

Disclosures: C.A. Zambrano: None. **D. Escobar:** None. **J.A. Stitzel:** None.

Poster

682. Nicotinic Acetylcholine Receptors: Structure and Regulation

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 682.18/F53

Topic: B.02. Ligand-Gated Ion Channels

Support: ARC DP150103990

Title: Molecular determinants of α-conotoxin Vc1.1 inhibition of human α9α10 nAChR

Authors: *D. J. ADAMS[1], H.-S. TAE[1], R. YU[2];
[1]Illawara Hlth. and Med. Res. Inst. (IHMRI), Univ. of Wollongong, North Wollongong, Australia; [2]Sch. of Med. and Pharm., Ocean Univ. of China, Qingdao, China

Abstract: Nicotinic acetylcholine receptor (nAChR) α9α10 subtype mediates postsynaptic transmission in the auditory system and is expressed in sensory neurons, cancer cells, keratinocytes and immune cells. α-Conotoxin Vc1.1 a disulfide-bonded peptide from the venom of *Conus victoriae* is an antagonist of α9α10 nAChRs and a potent analgesic in rat neuropathic and chronic pain models. Two stoichiometries of the receptor, $\alpha9_2\alpha10_3$ and $\alpha9_3\alpha10_2$, have been proposed whereby at $\alpha9_3\alpha10_2$, Vc1.1 is postulated to bind at high sensitivity (HS) α9(+)α9(-) and low sensitivity (LS) α10(+)α9(-) interfaces. However, the molecular mechanism governing the interaction of Vc1.1 at α9α10 nAChR remains unclear. Given that both interfaces share the same α9(-) side, the difference in Vc1.1 sensitivity should be primarily contributed by non-conserved residues on (+) side of the subunits. We tested the hypothesis, derived from molecular modeling and computation docking of Vc1.1 at human α9α10 nAChR, that a non-conserved α9(+)N179 (previously N152) residue (G in α10(+)) is involved in Vc1.1 binding sensitivity. *Xenopus laevis* oocytes were injected with varying cRNA ratios of human α9 to α10 subunits (1:3 and 1:1) to putatively form the stoichiometries and ACh-evoked currents were recorded using two-electrode voltage clamp method. At a 1:1 ratio, the α9(+)N179G mutation reduced Vc1.1 potency due to the loss of Vc1.1-HS sites. In contrast, at 1:3 ratio favouring LS sites, Vc1.1 inhibited wild-type α9α10 nAChRs with reduced potency without affecting the sensitivity of α9(+)N179Gα10 nAChRs. In all cases, the decrease in Vc1.1 sensitivity is similar suggesting a stoichiometry of $\alpha9_3\alpha10_2$ in oocytes injected with 1:1 ratio of α9 to α10 subunits. In conclusion, the non-conserved N179 residue is essential in the formation of Vc1.1 HS binding sites at the α9(+)α9(-) interface in the postulated $\alpha9_3\alpha10_2$ stoichiometry.

Disclosures: **D.J. Adams:** None. **H. Tae:** None. **R. Yu:** None.

Poster

683. HCN, Cation, and Other Channels

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 683.01/G1

Topic: B.04. Ion Channels

Title: ion channels antibodys role in neuronal survival

Authors: ***N. AYSIT**;
neuroscience, ISTANBUL Medipol UNIVERSITY -ISTANBUL MEDIPOL UNIV, ISTANBUL, Turkey

Abstract: Neuronal excitability is finely controlled by various membranous channels and associated proteins. In the central nervous system (CNS), the rapidly inactivating voltage-gated potassium channels (Kv channels) are the major determinants of dendritic excitability. Voltage-

gated potassium channels (Kvs) play a critical role in regulating neuronal excitability and synaptic plasticity in the hippocampus. NMDA receptors, a type of Kv channel, are critical for the development of the CNS, generation of rhythms for breathing and locomotion, and the processes underlying learning, memory, and neuroplasticity. Consequently, abnormal expression levels and altered NMDAR function have been implicated in numerous neurological disorders and pathological conditions. NMDAR hypofunction can result in cognitive defects, whereas overstimulation causes excitotoxicity and subsequent neurodegeneration. Subconductance levels have been observed in virtually every type of ion channel, although the number of levels, stability, and abundance vary widely. Sublevels have been well-characterized in K channels, which are evolutionarily related to NMDARs. The aim of the study is the contribution of Kv and NMDA receptors of neuronal survival/death in hippocampal cells . Primary hippocampal cultures were prepared from the brains of newborn Bulb-c mice approximately 4 days old. The hippocampus was isolated from the brain of each mice and treated with at 4 °C. The cells were suspended in neurobasal medium and were plated at several densities 200 -1600 cells per mm2. IgG was isolated from healthy partıcıpantsand limbic patients who were NMDAR ,Kv channel pozitive. Differrent concentrations of Isolated IgG were added to the preperation at days 7v and 10. in vitro. Imagıng was performed on same days by confacal microscobe and dead cells were determined using propidium iodide. In this study T test was used for analyzes and shown that using high concentration NMDA and Kv IgG cause neuron and astrocyte death. These result showed that ion channels antibody is not only important for their function but also they regulate neuronal survival. Otherwise Neuron was deadth not only apoptozis but also necrozis.

Disclosures: **N. Aysit:** None.

Poster

683. HCN, Cation, and Other Channels

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 683.02/G2

Topic: B.04. Ion Channels

Support: NSF 1535790

Title: Exploring the thermosensitivity of Gustatory Receptors in *Drosophila melanogaster*

Authors: ***A. MISHRA**, A. ROBINSON, T. ZARS;
BIOLOGICAL SCIENCES- NEUROSCIENCE, UM-COLUMBIA, Columbia, MO

Abstract: The ability to detect shifts in temperature is vital for the survival of organisms. Other than the highly conserved thermosensitive Transient receptor potential (Trp) channels, little is

known about the molecules involved in temperature sensation in *Drosophila*. The Gustatory Receptor (GR) 28b-d helps flies respond to rapid changes in temperature. The discovery of temperature response properties of *GR28b-d* indicates the potential for other GRs as temperature responsive. We are studying the temperature responsiveness of the 68 GR genes in *Drosophila*, starting with *GR28* family. To test thermosensitivity, we use the heat box, which can detect the temperature preference of flies with a resolution of less than 1^{o}C. We over-expressed *GR28a* and the isoforms of *GR28b* pan-neuronally, using the *nSyb-GAL4* driver. The temperature in the heat box was increased in steps of 2^{o}C from 24-40^{o}C. At the temperature of activation of the mis-expressed gene, we expect a temporary paralysis of flies. Our preliminary results show that, out of the five isoforms of *GR28b*, only *GR28b-d* is thermosensitive in a temperature range of 34-36^{o}C. Additionally, we measured the time taken by the flies over-expressing *GR28b-d* to recover from temporary incapacitation following exposure to temperature stimulus of varying intensities. We discovered that flies that are exposed to a lower temperature stimulus (34^{o}C) recovered faster than flies that received a higher temperature stimulus (36-40^{o}C). We continue to screen the GRs for thermosensitive genes. Along with our collaborators, we are studying the molecular properties of the GRs in the *GR28b* family. Based on our results, we aim to develop novel thermogenetic tools that can act as molecular switches to enable finer temperature-dependent control of neuronal activity in different model systems.

Disclosures: A. Mishra: None. **A. Robinson:** None. **T. Zars:** None.

Poster

683. HCN, Cation, and Other Channels

Location: Halls B-H

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Program#/Poster#: 683.03/G3

Topic: B.04. Ion Channels

Support: Heart & Stroke Foundation of Canada

Title: Cell swelling and SLC26A11 during simulated ischemia in neocortical brain slices

Authors: H. EL-KERDAWY, K7L 3N6, J. CARR, *R. D. ANDREW;
Queen's Univ., Kingston, ON, Canada

Abstract: During the first few minutes of ischemia, neurons swell as they undergo anoxic depolarization (AD). Water transporters energetically linked to the failing Na/K pump lose their function and neuronal water accumulates. Passive water loss is slow because neurons lack aquaporins. One proposal is that neurons swell because depolarization evokes a large increase in Na^{+} influx with Cl^{-} following via SLC26A11, a Cl^{-} channel/transporter. Water then osmotically

follows these ions into neurons. We examined in live brain slices if slice swelling during simulated ischemia (O_2/glucose deprivation, OGD) requires SLC26A11-mediated chloride influx. Neuronal and astrocytic swelling during OGD-induced AD is indirectly imaged in live coronal brain slices as a front of elevated light transmittance (LT) spreading across the neocortex. We compared swelling and its time course at 34^{o}C during AD in a) regular artificial CSF; b) low-chloride aCSF (Cl^- replaced with isethionate, methyl sulfate or proprionate); c) regular aCSF + PPQ-102 or niflumic acid, two drugs that inhibit SLC26A11 function. In low Cl^- aCSF, the propagating AD increased in LT strength and AD onset was not delayed (n=12 slices) compared to regular aCSF (n=9). Apparently the larger anions easily substituted for Cl^- influx. Pre-treatment prior to OGD with 10 uM (n=15) or 20 uM (n=11) PPQ-102 did not delay nor reduce the AD-associated LT increase compared to 25 slices without PPQ-102. Likewise 100 uM niflumic acid pre-treatment (n=16) had so significant effect on AD-associated swelling onset nor intensity compared to regular slices under OGD. Niflumic acid pre-treatment did significantly reduce the exaggerated LT increase during OGD by ~25% when proprionate was substituted for Cl^- , but swelling onset was unaffected (n=10). Two-photon microscopy of 15 live pyramidal cells containing yellow fluorescent protein (YFP) confirmed that OGD-induced neuronal swelling was not significantly reduced by PPQ-102 or niflumic acid. So in this well-established model of simulated ischemia, SLC26A11-mediated chloride influx appeared to play a minor role in acute neuronal swelling.

Disclosures: H. El-Kerdawy: None. **J. Carr:** None. **R.D. Andrew:** None.

Poster

683. HCN, Cation, and Other Channels

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 683.04/G4

Topic: B.04. Ion Channels

Title: The calcium-activated chloride channel mediates afterhyperpolarization in thalamocortical neurons

Authors: *G. HA[1], J. LEE[2], H. KWAK[1], K. SONG[1], J. KWON[2], S.-Y. JUNG[2], J. HONG[1], G.-E. CHANG[1], E. HWANG[2], H.-S. SHIN[3], C. J. LEE[2], E. CHEONG[1];
[1]Yonsei Univ., Seoul, Korea, Republic of; [2]Korea Inst. of Sci. & Technol., Seoul, Korea, Republic of; [3]Inst. for Basic Sci., Daejeon, Korea, Republic of

Abstract: In central nervous system (CNS), afterhyperpolarization (AHP) activated after single or multiple action potentials in neurons plays an important role in the modulation of neuronal excitability by limiting firing frequency and thus in the generation of spike-frequency adaptation.

We previously reported that calcium-activated AHP currents in thalamocortical (TC) neurons were mediated by multiple components including apamin-sensitive SK channels and niflumic acid-sensitive anion channels whose molecular identity was unknown. Here we investigated the calcium-activated chloride channels (CACCs) mediating AHP in TC neurons. CACCs are involved in many physiological phenomena, but the functional expression and properties of them in CNS have rarely been reported. We characterized the electrophysiological properties of CaCCs in TC neurons and investigated its molecular identity.

Disclosures: **G. Ha:** None. **J. Lee:** None. **H. Kwak:** None. **K. Song:** None. **J. Kwon:** None. **S. Jung:** None. **J. Hong:** None. **G. Chang:** None. **E. Hwang:** None. **H. Shin:** None. **C.J. Lee:** None. **E. Cheong:** None.

Poster

683. HCN, Cation, and Other Channels

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Topic: B.04. Ion Channels

Support: National Natural Science Foundation of China (No. 81573405)

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National Basic Research Program of China (No. 2015CB553504)

Title: Brain hyperpolarization-activated cyclic nucleotide-gated channels modulate methamphetamine self-administration in rats

Authors: ***R. SONG**, D.-N. CAO, S.-Z. ZHANG, N. WU, J. LI;
Beijing Insistitute of Pharmacol. and Toxicology, Beijing, China

Abstract: *Rationale* Methamphetamine addiction is believed to primarily result from increased dopamine release and the inhibition of dopamine uptake. Some evidence suggests that the hyperpolarization-activated cyclic nucleotide-gated (HCN) channels play important roles in the functional modulation of dopaminergic neurons and the pathophysiology of related diseases. However, little is known about the effects of HCN channels on methamphetamine addiction. *Objectives* The present study investigated the role of brain HCN channels in methamphetamine addiction. *Results* Acute intracerebroventricular (i.c.v.) injection or bilateral intra-accumbens microinjections of non-selective HCN channel blocker ZD7288 (0.3125 and 0.625 μg) significantly reduced both the methamphetamine (0.0125 or 0.05 mg/kg/infusion)-induced self-administration under fixed ratio 2 reinforcement and the breakpoint of methamphetamine (0.05

mg/kg/infusion) under progressive ratio reinforcement in rats. Moreover, compared with the i.c.v. injection, bilateral intra-accumbens microinjections of ZD7288 exerted stronger inhibitory effects, suggesting that the blockade of HCN channels in the nucleus accumbens reduced the reinforcing effects of and motivation for methamphetamine. We also found that ZD7288 (0.625 and 1.25 μg, i.c.v.) significantly decreased methamphetamine (1 mg/kg, intraperitoneal (i.p.))-induced hyperactivity with no effect on the spontaneous activity in rats. Finally, *in vivo* microdialysis experiments showed that the HCN channel blockade using ZD7288 (0.625 and 1.25 μg, i.c.v.) decreased the methamphetamine (1 mg/kg, i.p.)-induced elevation of extracellular dopamine levels in the nucleus accumbens. *Conclusions* These results indicate that the HCN channels in the nucleus accumbens are involved in the reinforcing properties of methamphetamine and highlight the importance of HCN channels in the regulation of dopamine neurotransmission underlying methamphetamine addiction.

Disclosures: **R. Song:** None. **D. Cao:** None. **S. Zhang:** None. **N. Wu:** None. **J. Li:** None.

Poster

683. HCN, Cation, and Other Channels

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Topic: B.04. Ion Channels

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NIH R03MH101578

NASARD Independent Investigator Award 2015

KM201410028019

Title: Postnatal development of hyperpolarization activated current h current in pyramidal neurons & parvalbumin interneurons in mouse prefrontal cortex.

Authors: *S. YANG, JR[1,2], Y.-C. LI[1], P. YU[2], W.-J. GAO[1];
[1]Drexel Univ. Sch. of Med., Philadelphia, PA; [2]Capital Normal Univ. Sch. of Educ., Beijing, China

Abstract: The hyperpolarization-activated current (h-current or Ih) is an inward current generated by the opening of hyperpolarization-activated cyclic nucleotide-gated (HCN) cation channels. In the prefrontal cortex (PFC), Ih in pyramidal neurons is proposed to maintain persistent firing for the execution of working memory function; while in GABAergic

parvalbumin (PV)-containing interneurons, Ih is mainly involved in the generation of rhythmic activity. However, despite its critical role for these featured functions of the PFC, the biophysical properties of the Ih in pyramidal neurons versus PV interneurons in the developing PFC remains uncharacterized. Here we have studied the developmental changes of physiological properties of Ih in the PFC layer V pyramidal neurons and PV interneurons. Our data show that in pyramidal neurons, the steady state current of the Ih (Iss) was significantly increased from juvenile to adolescence and adult. Whereas the Iss recorded from adolescent group of PV interneurons was significantly smaller than that in pyramidal neurons. The kinetic of Ih in PV interneurons was significantly faster than that in pyramidal neurons. Ih also contributes to the intrinsic excitability of both pyramidal neurons and PV interneurons. In pyramidal neurons, the resting membrane potentials increased along with aging. A reduction of resting membrane potential caused by blocking Ih current was observed in adolescent and adult groups but not in juvenile group. The membrane input resistance also showed aging-related changes. Application of Ih blocker ZD7288 significantly increased input resistance in adolescent and adult animals, but not in juveniles. In the adolescent group of PV interneurons, the resting membrane potential was also significantly reduced by ZD 7288, but the input resistance did not show any change. These results indicate a developmental change of Ih in the PFC pyramidal neurons. The developmental changes of Ih in PV interneurons are still under investigation but they appear to be different from those observed in pyramidal neurons.

Disclosures: **S. Yang:** None. **Y. Li:** None. **P. Yu:** None. **W. Gao:** None.

Poster

683. HCN, Cation, and Other Channels

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Topic: B.04. Ion Channels

Support: ARC Discovery Project (EA; DP130101364)

ARC Centre of Excellence for Integrative Brain Function (CE140100007)

Title: Two-photon photoswitching of optovin induces neuronal activity in rat cortex

Authors: *E. KHERADPEZHOUH[1], J. M. C. CHOY[2], V. DARIA[1], E. ARABZADEH[1]; [1]Eccels Inst. of Neurosci., [2]Australian Natl. Univ., Acton, Australia

Abstract: Photoswitch molecules provide a well-controlled method for dissecting neuronal circuitries. Here, we characterize how two-photon photoswitching of optovin (a synthetic

molecule with photoswitch capability) triggers neuronal activity in rodent cortex and demonstrate that this activation is mediated via gating the Transient Receptor Potential Ankyrin 1 (TRPA1) as previously demonstrated in zebrafish[1]. We first established the presence of TRPA1 channel in cortical cells by immunohistochemistry and showed that TRPA1 was significantly expressed in neurons and vascular structures. To determine the functional effect of TRPA1 on cortical neurons, we conducted *in vitro* two-photon Ca^{2+} imaging (with Cal-520 AM) and examined the effect of TRPA1 activator (AITC, 1 mM) and inhibitor (HC-030031, 10 μM) on the free cytoplasmic Ca^{2+} concentration in layer 5 (L5) pyramidal neurons. Introducing 1 mM AITC to the bath solution resulted in a significant increase in $\Delta F/F$ which consequently returned to baseline with 10 μM HC-030031. To further confirm this finding, we performed *in vitro* whole-cell recordings of L5 pyramidal neurons and measured the membrane potential (V_m) in the presence of TRPA1 activator and inhibitor. Application of 1 mM AITC increased the V_m significantly (depolarization, n=3, $p < 0.0001$); by replacing the AITC with 10 μM HC-030031, V_m values returned towards baseline levels. Next, we characterized the optical control of neuronal activity by focal illumination of optovin-loaded neurons using a femtosecond laser. We performed whole-cell recording of L5 pyramidal neurons using an internal solution containing optovin. Consistently across neurons, the maximum neuronal response was detected at 720-nm two-photon photoswitching of optovin (n=17). By increasing the concentration of optovin ΔV_m increased significantly resulting in action potential firing in some neurons. We further loaded neurons with different concentrations of optovin (1-20 μM) and found a systematic enhancement of neuronal activity as the optovin concentration was increased. Finally, to confirm that optovin activated these neurons via specific interaction with TRPA1, we repeated the whole-cell recordings in the presence or absence of the highly selective TRPA1 blocker, HC-030031. In the presence of HC-030031, neuronal activity was significantly reduced ($p < 0.0001$). Our results indicate that two-photon photoswitching of optovin might provide a powerful method for investigating the neuronal circuitries in the mammalian cortex. Reference: 1. Kokel *et al.* (2013) Photochemical activation of TRPA1 channels in neurons and animals. *Nat Chem Biol* 9:257-263.

Disclosures: E. Kheradpezhouh: None. **J.M.C. Choy:** None. **V. Daria:** None. **E. Arabzadeh:** None.

Poster

683. HCN, Cation, and Other Channels

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Program#/Poster#: 683.08/G8

Topic: B.04. Ion Channels

Title: Regulation of mouse HCN2 channel surface expression through SUMO post-translational modification

Authors: *M. WELCH, L. A. FORSTER, A. R. PARKER, D. J. BARO;
Biol., Georgia State Univ. Dept. of Biol., Atlanta, GA

Abstract: The hyperpolarization-activated current (I_h) is a subthreshold conductance that influences a variety of intrinsic cellular properties including membrane potential, temporal summation and pacemaker activity. As such, I_h controls neuronal activity, and not surprisingly, neuronal activity regulates I_h over multiple time scales and through multiple mechanisms. In particular, activity is known to adjust the surface expression of Hyperpolarization-activated Cyclic Nucleotide-gated (HCN) ion channels that mediate I_h. Recently, the Small Ubiquitin like Modifier (SUMO) has emerged as a mechanism for regulation of ion channel surface expression. SUMO is an ~11kDa peptide that is post-translationally added to a lysine residue in a consensus sequence on target proteins. We identified several putative evolutionarily conserved SUMOylation sites on HCN channels using SUMOplot and have begun to test the hypothesis that SUMOylation mediates activity-dependent surface expression of mouse HCN2 channels. SUMOylated proteins were immunoprecipitated (IP) from adult mouse brain protein lysates and transferred to western blots that were probed with antibodies against mouse HCN2 channels. These experiments demonstrated that HCN2 channels were post-translationally modified by both SUMO1 and SUMO2 *in vivo*. Furthermore, human embryonic kidney (HEK) cells stably expressing mouse HCN2 (HEK-HCN2) showed a statistically significant roughly two-fold increase in HCN2 SUMOylation when the cell line was transiently co-transfected with plasmids encoding SUMO, its conjugating enzyme Ubc-9, and mCherry relative to transfection with mCherry alone, as determined with IPs followed by western blot experiments. Whole cell patch-clamp recordings from HEK-HCN2 cells demonstrated that I_h maximal conductance was significantly increased approximately two-fold in cells transiently transfected with SUMO, Ubc9, and mCherry relative to mCherry alone. There was no change in the voltage dependence or kinetics of activation. Biotinylation assays on HEK-HCN2 cells showed increased HCN2 surface expression in cells transiently transfected with SUMO, Ubc9, and mCherry relative to mCherry alone. We have mutated putative HCN2 SUMOylation sites that are highly conserved across species, individually and in combination, and are currently assaying the extent of HCN2 SUMOylation and I_h regulation in these mutants. Based on present data, we suggest that SUMOylation can regulate HCN2 surface expression, and future experiments will be designed to test whether or not neuronal activity can regulate HCN2 channel SUMOlyation.

Disclosures: **M. Welch:** None. **L.A. Forster:** None. **A.R. Parker:** None. **D.J. Baro:** None.

Poster

683. HCN, Cation, and Other Channels

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Program#/Poster#: 683.09/G9

Topic: B.04. Ion Channels

Title: Dorso-ventral gradient in hyperpolarisation-activated current (I_h) in hippocampal ca1 OLM-interneurons

Authors: *M. M. HILSCHER[1,2,3], F. RATTAY[3], K. KULLANDER[2], K. E. LEÃO[1], R. N. LEÃO[1,2];
[1]Federal Univ. of Rio Grande do Norte, Brain Inst., Natal, Brazil; [2]Dept. of Neurosci., Uppsala Univ., Uppsala, Sweden; [3]Inst. for Analysis and Scientific Computing, Vienna Univ. of Technol., Vienna, Austria

Abstract: Oriens-lacunosum moleculare (OLM) cells are a class of hippocampal interneurons known to gate information arising from the entorhinal cortex and CA3 in CA1. These neurons are have been shown to express somatostatin (Som+) and the cholinergic receptor, nicotinic, alpha 2 (Chrna2+), being the latter gene the most specific OLM marker in the intermediate/ventral CA1. OLM cells are also important players in the generation of theta oscillations. These neurons show strong spike phase lock with theta oscillation and modeling studies have suggested that OLM cells can synchronize clusters of pyramidal cells and basket interneurons.
We perfomed whole-cell current- and voltage-clamp recordings in the dorsal and ventral hippocampus and show that passive and active electrophysiological properties of OLM cells differ along the dorsal to ventral axis. Dorsal OLM cells generated more hyperpolarization-activated current (I_h) than ventral OLM cells, indicating a difference in the expression of the hyperpolarization-activated cyclic nucleotide-gated (HCN) channels. Furthermore, both dorsal and ventral OLM cells showed electrical resonance at theta frequencies, with dorsal OLM cells resonating in distinct frequency bands than ventral OLM cells. The resonance frequency was extracted from the impedance amplitude profile, i.e. the voltage response to a Chirp current stimulus, which consisted of a linearly increasing (1 Hz/s) sine wave with constant amplitude. This protocol was run under the application of tetrodotoxin to block action potentials. While at near-threshold potentials I_h was not active, at hyperpolarized potentials this current contributed to theta-resonance and could be suppressed with the HCN channel blocker ZD7288.
In summary, our data suggests that dorsal OLM cells differ from ventral OLM cells through the generation of I_h and that this current contributes to the electrical resonance in OLM cells at theta frequencies.

Disclosures: **M.M. Hilscher:** None. **F. Rattay:** None. **K. Kullander:** None. **K.E. Leão:** None. **R.N. Leão:** None.

Poster

683. HCN, Cation, and Other Channels

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 683.10/G10

Topic: B.04. Ion Channels

Support: NIMH Grant F32103964-01

Title: Learned fear induces a reduction in cerebellar stellate cell hyperpolarization-activated currents

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

Abstract: Conditioned fear is a form of associative learning that has been shown to cause changes in the cerebellar cortex in a lobule-specific manner. To date, relatively little is known about the influence of learning on membrane excitability. Hyperpolarization activated cyclic nucleotide-regulated (HCN) channels are nonselective cation channels that activate on membrane hyperpolarization and deactivate on depolarization. Since these channels are activated close to a neuron's resting membrane potential, they play a role in determining intrinsic membrane properties. Cerebellar stellate cells are inhibitory interneurons that express HCN, an alteration of which could influence how these cells respond to input, as well as what they output onto Purkinje cells. We previously found that fear conditioning (FC) reduces hyperpolarization-activated current (I_h) amplitude, causing an increase in neuronal input resistance. Further, we observed that FC-induced changes in I_h are reversed following extinction and that they are specific to vermal lobules V/VI. In the current study, we verified that basal levels of I_h in stellate cells from naïve animals in lobule IX were similar to lobules V/VI. This was important to confirm as previous studies have shown intra-lobule variability in neuronal intrinsic membrane properties. Next, we focused on the functional consequences of modifications in I_h by evaluating the input/output relationship of cerebellar stellate cells after FC. In order to investigate this, we stimulated parallel fibers in the molecular layer at various intensities and recorded resulting extracellular stellate cell activity. Using this approach, we observed a significant difference in the threshold at which cells from FC animals responded to parallel fiber stimulation. Moreover, when comparing the response of cells to the same stimulation intensity, FC cells fired at higher frequencies than cells from naïve animals. Finally, we investigated whether FC-induced changes in I_h would enhance the integration of hyperpolarizing inputs by examining the spike frequency

response of stellate cells to a series of negative, bidirectional current ramps. In cells recorded from naïve animals, blockade of I_h resulted in a more hyperpolarized membrane potential, as well as an increase in instantaneous frequency when spiking resumed at the end of the negative current injection. Similarly, FC cells exhibited greater action potential frequency just after cessation of the hyperpolarizing ramp, and this was presumably due to a reduction in I_h. Taken together with our previous findings, these data contribute to the understanding of how fear memories are encoded in the cerebellum.

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

Poster

683. HCN, Cation, and Other Channels

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Topic: B.04. Ion Channels

Support: NS084473

MH100510-01A1

2014 NARSAD Young Investigate Grant #22745 (funded by John Kennedy Harrison)

Title: An increase in somatic I_h correlates with depression-like states

Authors: *C. KIM, D. H. BRAGER, D. JOHNSTON;
The Univ. of Texas At Austin, Austin, TX

Abstract: The hippocampus is an integral brain region for affective disorders. We previously demonstrated that reduced expression of the HCN1 subunit of h-channels, and subsequent reduction in I_h, in the dorsal CA1 region leads to anxiolytic- and antidepressant-like effects. In light of this, we asked whether h-channel subunit expression and I_h are altered in an animal model of depression. Given that chronic stress can be a precipitating factor for the onset of depression, we used chronic unpredictable stress (CUS) to test the hypothesis that HCN1 protein expression and I_h are elevated following CUS. We found that HCN1 protein expression was significantly increased in the dorsal, but not ventral CA1 region following CUS. In addition, we found that somatic, but not dendritic, input resistance was lower and resonant frequency higher following CUS, consistent with higher somatic I_h. In agreement, cell-attached patch clamp recordings revealed somatic I_h was significantly higher in dorsal CA1 neurons following CUS. Surprisingly, when dorsal CA1 I_h is reduced by shRNA-HCN1, the CUS-induced depressive behavioral phenotypes (e.g. anhedonia and behavioral despair) are reversed. Our results suggest

that CUS induces a functional increase in somatic I_h in dorsal CA1 neurons and furthermore that reduction of dorsal CA1 HCN1 protein expression is sufficient to produce resiliency to CUS.

Disclosures: **C. Kim:** None. **D.H. Brager:** None. **D. Johnston:** None.

Poster

683. HCN, Cation, and Other Channels

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Program#/Poster#: 683.12/G12

Topic: B.04. Ion Channels

Title: Ih in CA1 pyramidal cell distal dendrites contribute to theta oscillation *In vitro*

Authors: ***M. D. LIMA**[1], S. MIKULOVIC[2], R. LEÃO[1,2];
[1]Brain Inst., UFRN, Natal, Brazil; [2]Uppsala Univ., Uppsala, Sweden

Abstract: The 4-12 Hz brain oscillation known as theta oscillation have been associated with movement when generated in the dorsal hippocampus and anxiety-like behaviours in the ventral hippocampus. We have previously shown that in horizontal hippocampus slices (from the ventral/intermediate hippocampus), OLM interneurons targeting distal dendritic compartments of CA1 pyramidal cells (PC) can modulate theta oscillations. In this work, we investigated the pyramidal cell membrane properties that facilitate theta generation by dendritic targeting interneurons. We first found that high density of Ih in distal apical dendrites of PCs produced rebound dendritic action potentials following OLM inhibition. Using pressure ejection of ZD7288 to block distal Ih, we found that OLM interneuron stimulation can no longer generate theta oscillations in slices. Taken together, our data show that an interplay between OLM interneurons and PC is crucial to theta generation. Also, we show that Ih in distal dendrites is crucial to theta rhythmogenesis.

Disclosures: **M.D. Lima:** None. **S. Mikulovic:** None. **R. Leão:** None.

Poster

683. HCN, Cation, and Other Channels

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Program#/Poster#: 683.13/G13

Topic: B.04. Ion Channels

Support: NIH NS069689

Title: Function and localization of HCN channels on oriens-lacunosum moleculare cells

Authors: Y. Y. LOPEZ[1,3], M. SOLIS-WHEELER[1,2], V. SEKULIC[5], M. KOZHEMYAKIN[3], J. MANRING[3], S. MIKULOVIC[6], K. KULLANDER[6], R. LEAO[6,7], F. SKINNER[5,8,9], *J. J. LAWRENCE[3,4];
[1]Dept. of Biol., [2]Honors Col., Texas Tech. Univ., Lubbock, TX; [3]Dept. Pharmacol. and Neurosci., [4]Ctr. for Translational Neurosci. and Therapeut., Texas Tech. Univ. Hlth. Sci. Ctr., Lubbock, TX; [5]Univ. Hlth. Network, Krembil Res. Inst., Toronto, ON, Canada; [6]Dept. of Neurosci., Uppsala Univ., Uppsala, Sweden; [7]Inst. do Cerebro, Univ. Federal do Rio Grande do Norte, Natal, Brazil; [8]Dept. of Physiol., [9]Med. (Neurology), Univ. of Toronto, Toronto, ON, Canada

Abstract: Septohippocampal circuits generate theta rhythms that are associated with hippocampal learning. CA1 oriens lacunosum-moleculare (O-LM) cells receive robust GABAergic input from parvalbumin-positive (PV) projection neurons located in the medial septum-diagonal band of Broca (MS-DBB; Borhegyi et al., 2004). Multi-compartment computational models of O-LM cells have shown differential effects of somatic versus dendritic expression of hyperpolarization-activated cyclic nucleotide-gated (HCN) channels on output features such as back-propagating action potentials elicited by synaptic inputs (Sekulić et al., 2014, 2015). Preliminary modeling results demonstrate that driving synchronous PV MS-DBB synaptic input at theta frequency recruits HCN channels, enabling theta frequencies to be transmitted through O-LM cells via rebound spiking. Therefore, defining the subcellular distribution of HCN channels on O-LM cells is critical in understanding how O-LM cells integrate and transmit PV MS-DBB synaptic input. Previous studies employing HCN1/HCN2 double knockout mice indicated the presence HCN1-and HCN2-containing channels on O-LM cells (Matt et al., 2010). HCN2-containing channels were localized to the somata of somatostatin-positive cells in the CA1 stratum oriens (Matt et al., 2010), but the distribution of HCN2 channels on O-LM cell dendrites has neither been examined nor defined. Here, we employed Chrna2-CRE/tdTomato mice, enabling tdTomato to be visualized in all intracellular compartments of O-LM cells (Mikulović et al., 2015). In accordance with previous observations (Matt et al. 2010), HCN2 labeling was largely restricted to the somata of O-LM cells. HCN2 labeling in O-LM cell dendrites revealed a decrease in HCN2 channel expression with

decreasing distance from the soma. However, we also observed somatic labeling in CA1 pyramidal cells, with HCN2 labeling being weakest in the CA1 LM layer. Therefore, the axons of O-LM cells are unlikely to contain significant HCN2 channel densities. Moreover, given previous observations localizing HCN1 channels to the distal dendrites of CA1 pyramidal cells (Lorincz et al. 2002), HCN2 channels may have a more proximal subcellular distribution both in principal cells and interneurons. A better understanding of the subcellular localization of HCN1 and HCN2 channels will provide insight into circuit dysfunction as a consequence of their improper recruitment by altered synaptic input and the implications of such mechanisms in several neurological disorders, such as epilepsy and Alzheimer's disease.

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Poster

684. Electrical Synapses and Gap Junctions

Location: Halls B-H

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Topic: B.07. Synaptic Transmission

Support: Goettingen Graduate School for Neurosciences, Biophysics and Molecular Biosciences (GGNB) Excellence Stipend

Title: Role of Piccolo in high frequency transmission at a central auditory synapse

Authors: *T. BUTOLA[1,2], T. MOSER[1,2];
[1]Max Planck Inst. For Biophysical Chem., Goettingen, Germany; [2]Inst. for Auditory Neurosci., Goettingen, Germany

Abstract: Piccolo is a cytomatrix of the active zone (CAZ) protein involved in scaffolding and regulating neurotransmitter release at neuronal active zones. Here, we used Piccolo mutant mice to study central auditory synapses of the cochlear nucleus, which are specialized in high throughput synaptic transmission, capable of sustaining signaling frequencies of several hundreds of Hertz. We argue that even subtle deficits in the regulation of vesicle dynamics and neurotransmitter release will be revealed at synapses with such high functional demands. Moreover, the signal this synapse receives is unbiased by the mutation due to the presence of an unaffected, short isoform of Piccolo (i.e. 'Piccolino') upstream at the ribbon synapses of cochlear inner hair cells. Hence, this site of investigation provides a unique opportunity to study the implications of Piccolo deficiency on neuronal synaptic transmission. At the endbulb of Held

synapse, we observed faster rise of miniature excitatory postsynaptic currents (mEPSCs) in the mutants, while mEPSC amplitude and frequency were unchanged. Likewise, we found a faster rise of evoked EPSCs in the mutants. Moreover, when stimulated with high frequency train stimulation, the mutant responses showed faster kinetics of depression and an increased probability of vesicle release, while estimates of pool size and vesicle replenishment remained unchanged. Faster kinetics of release may be due to faster presynaptic release machinery with tighter Ca^{2+} influx and exocytosis coupling. Our hypothesis puts Piccolo as a spacer between the readily releasable pool of vesicles and Ca^{2+} channels, regulating release. Current experiments aim at revealing the contribution of postsynaptic receptor desensitization and saturation, to the observed synaptic changes. We also plan to address changes in the abundance of other CAZ proteins at the Piccolo-deficient endbulb active zones and study effects of combined manipulation of Piccolo and Bassoon.

Disclosures: **T. Butola:** None. **T. Moser:** None.

Poster

684. Electrical Synapses and Gap Junctions

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Topic: B.07. Synaptic Transmission

Support: Reinhart-Koselleck Grant of the German Research Foundation

Title: Common mechanisms of electrical synapse plasticity in invertebrates and vertebrates

Authors: ***G. WELZEL**, S. SCHUSTER;
Animal Physiol., Univ. of Bayreuth, Bayreuth, Germany

Abstract: Electrical synapses formed by gap junctions are of fundamental functional importance in the nervous system. Invertebrates form these gap junctions by using the supposedly primordial innexin proteins, whereas vertebrates use a different and unrelated protein family, the connexins. Vertebrates additionally express innexin-homologs in large quantities but only recruit connexin-based synapses for neuronal processing. These synapses have become renowned for their dynamic and use-dependent plasticity. Comparable data are lacking for innexin-based synapses so that it is unknown whether the two families differ fundamentally in how they support use-dependent plasticity. Here we directly studied the *in vivo* conductance in an innexin-based electrical synapse that is important for modulating escape behavior in the leech (*Hirudo medicinalis*). Our results show unequivocally that this innexin-based synapse shares all hallmarks of dynamic and use-dependent plasticity that are known for connexin-based synapses

of vertebrates. We show that serotonin and dopamine efficiently and rapidly modulate conductance with time constants of 14 - 18 and 48 s, respectively. Moreover, we demonstrate for the first time in any innexin-based synapse that these are capable of stable long-term potentiation (LTP) and may even share fundamental mechanisms of induction with their vertebrate counterparts. Our findings suggest that electrical synapse plasticity may be a conserved property of ancestral gap junctions that is shared by innexin- and connexin based synapses.

Disclosures: **G. Welzel:** None. **S. Schuster:** None.

Poster

684. Electrical Synapses and Gap Junctions

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Topic: B.07. Synaptic Transmission

Support: NSF RTG DMS1344962

Title: The role of electrotonic junctions between excitatory neurons in the cortex

Authors: ***J. KILE**[1], G. KOVACIC[1], D. CAI[2];
[1]Rensselaer Polytechnic Inst., Troy, NY; [2]Courant Institute, NYU, New York, NY

Abstract: Global oscillations in the brain are linked to synchronized neuronal activity, which has been shown to contribute to cognitive processes such as perception, motor performance, learning and memory. Electric coupling through gap junctions may facilitate the emergence of synchronized oscillations, and influence their properties. Gap junctions between inhibitory neurons in the mammalian cerebral cortex have been well studied, but electrical synapses between excitatory, pyramidal neurons, or electrotonic junctions, have only recently been discovered experimentally.
In this study, we closely follow experimental data to construct a detailed, comprehensive model with both synaptic and electric coupling for both excitatory and inhibitory neurons using a modified version of the Hodgkin-Huxley equations. We have successfully replicated data for the interaction between pairs of electrically connected excitatory neurons, and pairs of electrically connected inhibitory neurons, using our mathematical model.
Our realistic, computational network model includes 25% inhibitory neurons and 75% pyramidal cells coupled both electrically and synaptically. We use a 5% coupling probability for gap junctions to occur among neighboring pyramidal cells, and a 50% coupling probability for gap junctions between interneurons less than 80μm apart, mimicking the medial prefrontal cortex, and visual cortex, of rats and ferrets.

We organize the neurons on a grid to capture the highly structured spatial properties of a network containing both synaptic and gap-junction connections, and to ensure that the probability of neurons being coupled is dependent on their location within the network. The external input to this patch of the cortex, modeling the influences of neurons from other parts of the brain, is in the form of a Poisson spike train. Using this model, we examine the dynamical regimes resulting from the inclusion of both electric and synaptic connections, with a specific interest in the emergence and properties of synchrony.
We find that the addition of gap junctions between inhibitory neurons creates oscillations in the firing pattern of the inhibitory population. Further, we show that these oscillations are strictly due to the gap junctions rather than an increased synaptic current. We also find that the pairs of electrotonic junctions serve to create specific patterning in the firing and bursting of the excitatory neurons that is linked to the timing of the inhibitory neurons' oscillations.

Disclosures: **J. Kile:** None. **G. Kovacic:** None. **D. Cai:** None.

Poster

684. Electrical Synapses and Gap Junctions

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 684.04/G17

Topic: B.07. Synaptic Transmission

Title: Mechanisms of PKA-Dependent Regulation of Tomosyn

Authors: ***S. J. ZINN**[1], J. E. RICHMOND[2], D. FEATHERSTONE[2];
[2]Biol. Sci., [1]Univ. of Illinois, Chicago, Chicago, IL

Abstract: Tomosyn, a presynaptic inhibitor of neurotransmitter release, forms non-fusogenic SNARE complexes with the plasma membrane SNAREs, syntaxin and SNAP-25. In vertebrates, PKA-dependent tomosyn phosphorylation disrupts its interaction with the SNAREs *in vitro*. This has yet to be demonstrated in the intact nervous system. Our previous studies in *Drosophila* have shown that both tomosyn knockdown and forskolin application enhance neurotransmission although these effects are not additive, suggesting they act in the same pathway. Tomosyn knockdown flies also exhibit impaired PKA-dependent olfactory learning, indicating that tomosyn may be a downstream target in this modulatory pathway. However, synapsin has also been implicated in fly PKA-dependent learning. This raises the possibility that PKA-regulation of tomosyn may be a secondary consequence of synapsin phosphorylation. To address this hypothesis, we have obtained a fly line expressing beggiatoa photoactivated adenylyl cyclase (bPAC), an optogenetic tool that allows blue-light activation of cAMP. At the fly NMJ, activation of bPAC results in enhanced neurotransmitter release, translocation of tomosyn away

from the plasma membrane, and a corresponding increase in synaptic vesicle docking by EM. To test whether PKA-regulation of tomosyn is direct or indirect, we are exploring the effect of synapsin mutations on cAMP-dependent tomosyn translocation. We are also generating a tomosyn-HA line using CRISPR/Cas9 to identify physiologically relevant tomosyn phosphorylation sites by mass spectrometry. Identification and subsequent mutation of potential PKA phosphorylation sites will allow us to directly test the relevance of tomosyn PKA-phosphorylation through site directed mutagenesis.

Disclosures: **S.J. Zinn:** None. **J.E. Richmond:** None. **D. Featherstone:** None.

Poster

684. Electrical Synapses and Gap Junctions

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Topic: B.07. Synaptic Transmission

Support: Conacyt CB-2010-01-0154645

Title: Glutamatergic & Dopaminergic neurons in the Substantia nigra of adult mouse preferentially express connexin 26 and 30

Authors: *A. HERNANDEZ SANCHEZ;
Univ. Autonoma De San Luis Potosi, San Luis Potosi, Mexico

Abstract: The mouse substantia nigra contains neurons of Dopaminergic (TH+, DAT+ or VMAT2+), Glutamatergic (VGluT2+) and GABAergic (GAD1/2+ or VGAT+) phenotypes as well as neurons of Dopaminergic/GABAergic (TH+/GAD+) and Dopaminergic/Glutamatergic (TH+/VGluT2+) double phenotype. Dopamine neurons communicate with neighbor neurons through chemical neurotransmission via the somatodendritic release of dopamine. Also, dopamine neurons synchronize during phasic electrical activity, which is modulated by electrical synapses. Interestingly, during both appetitive and aversive learning, non-dopaminergic neurons show synchronized electrical activity with dopaminergic neurons. In order to gain insight into this phenomenon, the expression profile of connexins, the building blocks of gap junctions which are responsible for fast neuronal electrical coupling, was determined in individual Dopaminergic (TH+/VGluT2-) and Glutamatergic (TH-/VGluT2+) neurons of the substantia nigra of adult (p90) mice. Single cell RT-PCR multiplex assays performed using isolated acutely-dissociated cells showed that both Dopaminergic and Glutamatergic cells can express Cx26, Cx30, Cx31.1, Cx43 and Cx45. Of them, Cx26 and Cx30 are expressed in the vast majority of these cells. Moreover, despite previous reports of the expression of Cx36 in dopamine neurons from young

mice, its expression couldn´t be detected in neurons from the adult substantia nigra. Given that Cx26 and Cx30 have the ability to form functional gap junctions either together or in combination, our results open the possibility of an electrical coupling between neurons of a different phenotype within the substantia nigra. The physiological meaning of this electrical coupling is currently under investigation in our laboratory.

Disclosures: A. Hernandez Sanchez: None.

Poster

684. Electrical Synapses and Gap Junctions

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DoD CDMRP, W81XWH-12-1-0187

Title: Tsc1 deletion in the developing thalamus synchronizes activity in relay neurons via ectopic electrical synapses

Authors: *R. MARTINEZ-GARCIA, S. R. CRANDALL, S. L. PATRICK, B. VOELCKER, M. ZERVAS, B. W. CONNORS;
Brown Univ., Providence, RI

Abstract: Tuberous Sclerosis (TSC) is a developmental genetic disorder caused by mutations in *Tsc1* and/or *Tsc2*. Neurological signs in TSC patients include epilepsy and autism, but the cellular mechanisms underpinning TSC are poorly understood. One brain region implicated in the pathology of some TSC patients is the thalamus. The laboratory previously generated a mouse model in which *Tsc1* was selectively deleted in ~70% of relay neurons on E12.5 (Normand et al., *Neuron*, 78:895, 2013). This deletion led to enlarged relay neurons with abnormal intrinsic excitability, and anomalous circuitry and synchrony in the thalamus; the deletion was also sufficient to cause aberrant repetitive grooming and seizures. One way to enhance neuronal synchrony is to interconnect cells with electrical synapses, which are comprised of neuronal gap junctions: intercellular channels that provide conductive pathways from one cell to another.

Surprisingly, we found strong but sparse electrical synapses between *Tsc1*$^{\Delta/\Delta}$ relay neurons in slices of the ventrobasal (VB) nucleus of the thalamus (*Tsc1*$^{\Delta/\Delta}$ mice: ~12% of cell pairs, *Tsc1*$^{+/+}$ mice: 0% of pairs; ages P20-P24). The laboratory has previously shown that gap junction-mediated electrical synapses are present in VB relay neurons early in development but are entirely abolished by the second postnatal week (Lee et al., *J. Physiol,* 588:2403, 2010). Electrically coupled *Tsc1*$^{\Delta/\Delta}$ relay neurons had synchronized subthreshold and spiking activity. Additionally, inhibitory inputs from the thalamic reticular nucleus to one thalamic relay neuron could, via electrical synapses, delay the activity of an adjacent neuron and briefly synchronize the activity of the cell pair afterwards. We also addressed whether *Tsc1* deletion in relay neurons alters chemical synapses. Relay cells with *Tsc1* deletions had a lower frequency of miniature excitatory postsynaptic currents, although the kinetics of these events were unaltered. Additionally, TRN inputs to *Tsc1*$^{\Delta/\Delta}$ relay neurons had slightly altered kinetics but unaffected synapse dynamics. Interestingly, *Tsc1*$^{\Delta/\Delta}$ relay neurons received more frequent feedback inhibition than control relay neurons.
Prenatal *Tsc1* deletions led to the induction ectopic electrical synapses among relay neurons as well as to changes in intrathalamic chemical synapses. These cellular changes may enhance synchrony among relay neurons and the cortex they project to, and contribute to repetitive grooming and seizures.

Disclosures: **R. Martinez-Garcia:** None. **S.R. Crandall:** None. **S.L. Patrick:** None. **B. Voelcker:** None. **M. Zervas:** None. **B.W. Connors:** None.

Poster

684. Electrical Synapses and Gap Junctions

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Topic: B.07. Synaptic Transmission

Title: Ankyrin and CRMP regulate gap junction dynamics through kinesin in *C. elegans*

Authors: *L. MENG[1], C.-H. CHEN[1], D. YAN[1,2];
[1]Mol. Genet. and Microbiology, [2]Neurobio., Duke Univ. Sch. of Med., Durham, NC

Abstract: The importance of gap junctions has been documented in many biological processes, but the molecular mechanisms underlying gap junction dynamics remain unclear. Here, we use *C. elegans* PLM neurons as a model to study gap junctions. In a genetic screen, we isolated two mutants, *unc-44*/ankyrin and *unc-33*/CRMP (Collapsin Response Mediator Protein) altering gap junctions. Through genetic analysis and live imaging, we found that ankyrin and CRMP function

through a kinesin (VAB-8) in regulating gap junction dynamic. Therefore, we first show a signal pathway involved ankyrin, CRMP and kinesin in regulating gap junction dynamic.

Disclosures: **L. Meng:** None. **C. Chen:** None. **D. Yan:** None.

Poster

684. Electrical Synapses and Gap Junctions

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Topic: B.07. Synaptic Transmission

Support: NSERC PDF

NIH RO1 NS40296

Title: Regulation of dense core vesicle trafficking and fusion at *Drosophila* synapses

Authors: ***K. G. ORMEROD**, J. T. LITTLETON;
Picower Inst. for Learning and Memory, MIT, Cambridge, MA

Abstract: Two main classes of neurosecretory vesicles, synaptic vesicles (SVs) and dense core vesicles (DCVs), mediate regulated secretion in neurons. Fusion of SVs is a fast and highly regulated process, orchestrated by synaptic proteins that modulate docking, priming and fusion. While the molecular components driving SV fusion have been well characterized, less is understood about the molecular machinery underlying the trafficking and exocytosis of DCVs at synapses. DCVs transport, store and release proteins and neuropeptides at multiple sites, facilitating a wide range of biological processes including synaptogenesis, synaptic transmission, cell survival, and synaptic plasticity. Unlike the release of SVs, DCVs require a larger stimulus (30+ Hz stimulation) to trigger exocytosis. Given these considerable differences, we are interested in examining how the synaptic machinery that mediates trafficking and fusion of SVs and DCVs differ. We have generated tagged versions of DCV cargo and transmembrane components to investigate their trafficking at the *Drosophila* neuromuscular junction. We are also employing quantal resolution imaging of vesicle fusion at single active zones to determine how DCV containing neuromodulators regulate synaptic communication.

Disclosures: **K.G. Ormerod:** None. **J.T. Littleton:** None.

Poster

684. Electrical Synapses and Gap Junctions

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Topic: B.07. Synaptic Transmission

Support: SEP CONACYT 127658 MEXICO

Title: Expression and modulation of gap junction proteins in the adrenal gland during pre and perinatal development.

Authors: *E. M. PEREZ-ARMENDARIZ[1], A. ZARZA VELA[2], L. CRUZ-MIGUEL[2]; [1]DEPARTMENT OF TISSUE AND CELL BIOLOGY, NATIONAL AUTONOMOUS UNIVERSITY OF MEXICO, Mexico City, Mexico; [2]DEPARTMENT OF TISSUE AND CELL BIOLOGY, NATIONAL AUTONOMOUS UNIVERSITY OF MEXICO, Mexico, Mexico

Abstract: It is known that connexin 36 (Cx36) and connexin Cx43 (Cx43) are expressed in the adrenal gland in adult rodents. In addition, there is evidence that stress induced by cold, in adult animals, induces changes in these connexins. Moreover, intercellular channels have been involved in the release of catecholamines (CA) by chromaffin cells (CCs) under stress induced by intermittent hypoxia during labor. However, the molecular identity, spatial distribution and ontogeny of gap junctions in the CCs during prenatal development is unknown. In addition, it is ignored whether there are gap junction changes during the perinatal period. To investigate these aspects real-time PCR studies were performed using specific probes against Cx43 and Cx36 in preparations of adrenal gland pools at 13 and 18 days post coitum (dpc) as well as 3 days postpartum (dpp). Between 13 dpc and 3 dpp, an increase in mRNA levels was found for Cx36 (1.4 fold) and Cx43 (4 fold). These transcript elevations occurred correlated with an increase in N-methyl transferase phenyl (46 fold) and 3-β-hydroxy steroid dehydrogenase (7.5 fold) and cytochrome P450 family 11 subfamily β member 1 (3.5 fold) respectively. From above, Cx43 and Cx36 are genes of early expression in the adrenal gland that are up regulated in a development and functional dependent manner. The spatial distribution of the respective proteins at these stages is presently characterized. GRANT CONACyT-SEP no. 127658, Mexico.

Disclosures: E.M. Perez-armendariz: None. **A. Zarza Vela:** None. **L. Cruz-miguel:** None.

Poster

684. Electrical Synapses and Gap Junctions

Location: Halls B-H

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Program#/Poster#: 684.10/G23

Topic: B.07. Synaptic Transmission

Support: ANII URUGUAY

CSIC URUGUAY

Title: Electrical coupling and active membrane currents support lateral inhibition and lateral excitation between Mesencephalic Trigeminal (MesV) neurons

Authors: *F. DAVOINE[1], P. MONZÓN[1], S. CURTI[2];
[1]Facultad Ingeniería, Univ. De La República, Montevideo, Uruguay; [2]Facultad Medicina, Univ. De La República, Montevideo, Uruguay

Abstract: MesV neurons are primary afferents that innervate spindles of jaw closer muscles and mechanoreceptors of the periodontal ligament. Their cell bodies lie within the CNS and form part of the networks involved in the organization of orofacial behaviors. These neurons are electrically coupled through somatic Cx36-containing gap junctions and coupling is restricted to pairs or small clusters. Instead of the classical low-pass properties, electrical transmission at these contacts behaves as a band-pass filter with a peak near 50-80 Hz. Experimental evidence indicate that this characteristic results from the interaction of the passive properties of coupled neurons with the persistent Na+ current (INap) and an A-type K+ current (IA). This frequency transfer characteristic supports synchronization and coincidence detection between these cells suggesting that sets of MesV neurons defined by electrical coupling operate as functional units. However, little is known about the specific role of the INap and the IA in frequency selectivity and how these circuits of coupled neurons contributes to the processing of relevant sensory information. To address these questions we developed a computational model of MesV neurons using NEURON and Python. The model reproduces the morphological features of these neurons and the main active membrane conductances and was adjusted to fit several experimental parameters such as input resistance, time constant, action potential shape and spiking, using evolutionary multi-objective optimization. The numerical model was used to simulate pairs of coupled neurons to study in detail the role of the active membrane currents. We found that while INap improves the gain of transmission through amplification of subthreshold signals it tends to worsen the precision of synchronization. In contrast, while the IA have the opposite effect on the gain it reduces the time difference between pre- and postsynaptic spikes improving the precision of synchronization. Therefore, these two currents seems to play opposing roles in electrical transmission and their antagonistic actions results in a compromise between gain and precision to promote neuronal synchronization. Moreover, simulations and experiments show that coupled

MesV neurons differentiate coincident and non-coincident sensorial stimuli coming from their peripheral axons. Depending on the relative delay between inputs, afferent spikes facilitates activation of coupled cells (lateral excitation) or delays it (lateral inhibition). The extent of these opposing effects and the time window over which they operate are determined by the balance between the INap and IA.

Disclosures: F. Davoine: None. **P. Monzón:** None. **S. Curti:** None.

Poster

685. Long-Term Potentiation: Kinases and Intracellular Signaling

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 685.01/G24

Topic: B.08. Synaptic Plasticity

Support: Israel Science Foundation (ISF)

The United States-Israel Binational Science Foundation (BSF)

National Institute for Psychobiology in Israel (for ST and NN)

Title: A novel LTP mechanism that involves persistent self-activation of nitric oxide synthase (NOS)

Authors: A. TURCHETTI-MAIA[1], N. STERN-MENTCH[1,2], N. NESHER[1,2], T. SHOMRAT[1,2], *B. HOCHNER[1];
[1]Dept of Neurobiology, Hebrew Univ., Jerusalem, Israel; [2]The Ruppin Academic Center, Sch. of Marine Sci., Michmoret, Israel

Abstract: The *Octopus vulgaris* vertical lobe (VL) is organized as a simple feedforward fan-out fan-in network with a robust presynaptic, NMDA-independent, activity-dependent LTP at the fan-out synaptic layer. The octopus VL and its LTP are important for the acquisition of long-term memories outside the VL, as saturating LTP or severing the VL impaired long-term memory acquisition but did not erase older memories (Young and Boycott 1955, Shomrat et al 2008). In this work we started to investigate the mechanisms underlying VL LTP. We first show that LTP induction and maintenance are likely not to involve protein synthesis, as the administration of anisomycin 20μM in VL slice preparations, before or after LTP induction, did not block LTP induction nor maintenance for at least up to 10h after LTP induction. We then investigated whether NOS, a well-known mediator of synaptic plasticity in mollusks, is involved in the long-term maintenance of LTP. Indeed, the VL neuropil is heavily stained for NOS activity (NADPH-d). We found that at least up to 8h after tetanization induced LTP, NOS

inhibitors could block LTP expression as much as bringing the facilitated synaptic LFP back to its control amplitude. A complete block of LTP expression involved a reversal of the paired-pulse facilitation ratio to the pre-induction ratio, suggesting that blocking the LTP involves reversing the probability of transmitter release to the pre-LTP level. Usually the blocking effects were reversible and washing out the inhibitors brought the response back to a full LTP level. However, we found that as the time gets longer after LTP induction, the proportion of experiments where LTP did not recover increased and, interestingly, in these cases tetanization reinduced LTP. Taken together, these results suggest a novel "molecular memory switch" mechanism whereby activity-dependent NOS activation generates NO that retrogradely mediates LTP expression in the presynaptic terminals and at the same time maintains long-term activation of NOS. We hypothesize that such a molecular and protein synthesis-independent LTP may coordinate, through the VL output, a universal protein synthesis-dependent long-term memory acquisition at brain areas outside of the VL.

Disclosures: **A. Turchetti-Maia:** None. **N. Stern-Mentch:** None. **N. Nesher:** None. **T. Shomrat:** None. **B. Hochner:** None.

Poster

685. Long-Term Potentiation: Kinases and Intracellular Signaling

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Program#/Poster#: 685.02/G25

Topic: B.08. Synaptic Plasticity

Support: NIH/NINDS Grant 1F31NS086174

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NIH/NINDS Grant 5R01NS37112

Title: RGS14 - shutting down synaptic plasticity

Authors: ***P. R. EVANS**[1,2], P. PARRA-BUENO[1], M. ZHAO[4], D. J. LUSTBERG[4], J. J. YANG[5], N. T. SEYFRIED[3], P. GRIFFIN[6], S. M. DUDEK[4], R. YASUDA[1], J. R. HEPLER[2]; [1]Neuronal Signal Transduction, Max Planck Florida Inst. For Neurosci., Jupiter, FL; [2]Pharmacol., [3]Biochem., Emory Univ., Atlanta, GA; [4]Neurobio. Lab., Natl. Inst. of Envrn. Hlth. Sci., Research Triangle Park, NC; [5]Chem., Georgia State Univ., Atlanta, GA; [6]Mol. Therapeut., The Scripps Res. Inst., Jupiter, FL

Abstract: Pyramidal neurons in hippocampal area CA2 differ dramatically from neighboring CA3/CA1 pyramidal neurons in that synaptic long-term potentiation (LTP) is not as readily

induced. We previously identified Regulator of G Protein Signaling 14 (RGS14) as a critical brake on CA2 synaptic plasticity and learning and memory. RGS14 knockout (RGS14 KO) mice display a robust and nascent capacity for LTP in CA2 pyramidal neurons, which is absent in wild-type (WT) mice, and exhibit enhanced hippocampus-dependent learning and memory. However, the cellular mechanism(s) by which RGS14 suppresses LTP in CA2 remain unknown. The lack of plasticity in CA2 has been attributed to robust calcium (Ca^{2+}) buffering and extrusion mechanisms relative to CA3/CA1, but RGS14 has not been functionally linked to Ca^{2+}-activated signaling pathways critical for LTP induction. Therefore, we investigated whether RGS14 restricts LTP in hippocampal CA2 by regulating Ca^{2+}-stimulated pathways required for LTP.
To identify candidate signaling pathways through which RGS14 natively inhibits LTP in CA2, we first co-immunoprecipitated RGS14 from mouse brain. We find that RGS14 interacts with calmodulin (CaM), Ca^{2+}/CaM-dependent kinase II (CaMKII), and other members of Ca^{2+}-activated LTP signaling pathways. We validated these novel binding partners using biochemical assays and demonstrate that RGS14 directly binds CaM in a Ca^{2+}-dependent manner. Differential hydrogen/deuterium exchange (HDX) mass spectrometry reveals that Ca^{2+}/CaM binding to RGS14 causes significant conformational changes. Further, we show RGS14 binds to and is phosphorylated by CaMKII *in vitro*. We also find that viral expression of RGS14 in CA1 pyramidal neurons inhibits LTP induction, suggesting that RGS14 acts through pathways common to CA2 and CA1. Using field recordings and pharmacological inhibitors we find that the nascent CA2 LTP present in RGS14 KO mice requires NMDAR activation as well as CaMKII and PKA activity. We also demonstrate through two-photon glutamate uncaging experiments that RGS14 limits structural plasticity of dendritic spines in WT CA2 neurons whereas CA2 spines from RGS14 KO mice possess structural plasticity similar to CA1 controls. Ongoing studies using two-photon fluorescence lifetime imaging microscopy biosensors are further defining if RGS14 modulates postsynaptic Ca^{2+} levels, CaMKII activity, and other downstream signaling, to block plasticity in CA2. Together these studies provide novel mechanistic insight into the cellular regulation of synaptic plasticity in area CA2 and the key role RGS14 plays in this process.

Disclosures: **P.R. Evans:** None. **P. Parra-Bueno:** None. **M. Zhao:** None. **D.J. Lustberg:** None. **J.J. Yang:** None. **N.T. Seyfried:** None. **P. Griffin:** None. **S.M. Dudek:** None. **R. Yasuda:** None. **J.R. Hepler:** None.

Poster

685. Long-Term Potentiation: Kinases and Intracellular Signaling

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Topic: B.08. Synaptic Plasticity

Support: CNPq 202183/2015-7

FCT SFRH/BD/60301/2009

NSERC DG 418546-12

CIHR NIA 288936

Title: Pre- and postsynaptically expressed spike-timing-dependent plasticity contribute differentially to neural learning

Authors: *B. E. P. MIZUSAKI[1,2], S. S. Y. LI[1], R. P. COSTA[3], P. J. SJÖSTRÖM[1];
[1]Res. Inst. of MUHC, Montreal, QC, Canada; [2]Inst. de Física, Univ. Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil; [3]Univ. of Oxford, Oxford, United Kingdom

Abstract: After a long-standing debate over whether long-term plasticity is pre or postsynaptically expressed, the emerging consensus is that one or both are possible outcomes depending on factors such as age, induction protocol, and synapse type. It remains unclear, however, what specific computational impact there is to pre vs. postsynaptic expression. To address this, we systematically varied the locus of expression in a simplistic model of spike-timing-dependent plasticity (STDP) inspired by Song and Abbott (Nat Neurosci 2000 3:919; Neuron 2001 32:339): a single integrate-and-fire neuron received at least 100 excitatory inputs. Presynaptic changes were either implemented by altering the probability of release directly at a stochastic synaptic contact following a Binomial release model, or alternatively by changing short-term plasticity using the Markram-Tsodyks vesicle depletion paradigm. Postsynaptic changes were implemented by additive changes in the quantal amplitude. Synaptic weight changes were normalized to give rise to the same magnitude of change irrespective of whether probability of release or quantal amplitude was plastic. Learning was assessed as rate of convergence to a final steady state in two simple simulation frameworks testing either the coding of correlated inputs or of spiking response latencies. With the stochastic release model, plasticity of the probability of release led to faster convergence than plasticity of quantal amplitude did, irrespective of initial conditions. When modelling presynaptic expression of plasticity in terms of stochastic release, we thus found that the rate of learning was generally faster with presynaptic than with postsynaptic expression. However, with presynaptic expression implemented in terms of changes in short-term depression, the coding of correlated inputs was more rapid with postsynaptic than with presynaptic plasticity. This outcome could in part be ascribed to the details of the Song and Abbott STDP model, which is not tuned to the neurobiology of multiple spike pairings arising at high frequencies. A model biologically tuned to neocortical layer-5 pyramidal cell synapses (Costa et al, eLife 2015 4:e09457) is predicted to exhibit different properties. The vast majority of computational models rely on changing the synaptic gain to model synaptic plasticity, which corresponds to altering the quantal amplitude. This however is not a neutral choice, as it is in effect a bias toward postsynaptic expression. Here we explore key differences between pre and postsynaptic expression of STDP, demonstrating how this default assumption in modelling of plasticity may in fact affect simulation results.

Disclosures: **B.E.P. Mizusaki:** None. **S.S.Y. Li:** None. **R.P. Costa:** None. **P.J. Sjöström:** None.

Poster

685. Long-Term Potentiation: Kinases and Intracellular Signaling

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Topic: B.08. Synaptic Plasticity

Support: NIH Grant R01 MH097887

AHA Postdoctoral fellowship 16POST26560000

Title: Binding of calcium/calmodulin to PSD-95 N-terminus mediates homeostatic scaling down

Authors: ***D. CHOWDHURY**, M. L. TURNER, T. PATRIARCHI, M. MENDEZ, J. AMES, J. W. HELL;
Univ. of California Davis, Davis, CA

Abstract: The postsynaptic density scaffold protein PSD-95 is a key organizer of excitatory synapses. Abundance of glutamate receptors at the synapse, and thereby synaptic strength, is determined largely by synaptic PSD-95 abundance which, in turn, is regulated by activity. However, the mechanisms underlying the activity-induced regulation of PSD-95 synaptic localization are not clearly understood. Previous work from our lab has shown that influx of calcium following synaptic activation induces dispersal of PSD-95 from dendritic spines via binding of Ca^{2+}/Calmodulin (CaM) to the N-terminus of PSD-95 that reduces its palmitoylation (Zhang et al., *EMBO J*, 2014). The current study elucidated the role of PSD-95-CaM interaction in mediating removal of PSD-95 from synapses that accompanies homeostatic synaptic scaling down. Using peptide screening and NMR structural analysis, we identified E17 and T19 within PSD-95 N-terminus as the critical residues interacting with CaM. We generated CaM-binding-defective mutants (E17R and T19K) to test their role in PSD-95 mobilization seen in homeostatic scaling down. Using fluorescence microscopy in cultured hippocampal neurons overexpressing recombinant PSD-95, we observed that chronic elevation of network activity with the GABA-A receptor antagonist bicuculline reduced PSD-95 spine enrichment in spines and both mutants blocked this effect. Moreover, replacement of endogenous PSD-95 with either mutant blocked reduction of surface AMPA-type glutamate receptors (AMPARs) during scaling down. Further, charge inversion mutations of the interacting residues on CaM, compensating for PSD-95 mutations, restored scaling down of AMPARs. Further work is directed to verify the role of this interaction in regulating functional AMPARs using electrophysiological analysis. Reference: Y.

Zhang, L. Matt, T. Patriarchi, Z.A. Malik, D. Chowdhury, D.K. Park, A. Renieri, J.B. Ames, J.W. Hell 'Capping of the N-terminus of PSD-95 by calmodulin triggers its postsynaptic release', *The EMBO Journal* 33(12): 1341-53 (2014).

Disclosures: D. Chowdhury: None. **M.L. Turner:** None. **T. Patriarchi:** None. **M. Mendez:** None. **J. Ames:** None. **J.W. Hell:** None.

Poster

685. Long-Term Potentiation: Kinases and Intracellular Signaling

Location: Halls B-H

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Program#/Poster#: 685.05/G28

Topic: B.08. Synaptic Plasticity

Title: Plasticity of excitatory transmission onto hippocampal interneurons of the stratum lacunosum moleculare

Authors: *M. MERCIER, D. KULLMANN;
Inst. of Neurol., Univ. Col. London, London, United Kingdom

Abstract: Long-term potentiation (LTP) of excitatory transmission onto hippocampal principal cells is thought to play an important role in memory encoding. Within stratum radiatum, LTP at Schaffer collateral-CA1 pyramidal cell synapses is balanced by a complementary increase in the recruitment of feed-forward inhibitory interneurons (Lamsa *et al*., 2005). CA1 pyramidal cells also exhibit LTP at their distal synapses located in stratum lacunosum moleculare (SLM), which receive excitatory input from entorhinal cortex layer III (ECIII). Whilst this pathway recruits strong feed-forward inhibition, it is not known whether ECIII synapses onto SLM feed-forward interneurons can also be potentiated, and if so, what downstream effects this might have on pyramidal cell signaling.
Using whole-cell recordings from SLM interneurons in acute mouse hippocampal slices, we found that LTP could indeed be induced in these cells. Interestingly, this plasticity was NMDA-receptor dependent when induced by a low-frequency pairing protocol, but not when induced by a spike-timing-dependent-plasticity (STDP) protocol, and in all cases was not pathway-specific. Post-hoc anatomical characterization revealed at least a subset of LTP-expressing cells with neurogliaform cell-like morphology. Further experiments will probe the signaling pathways and mechanisms involved in this novel form of interneuron plasticity, as well as its function within the wider hippocampal network.

Disclosures: M. Mercier: None. **D. Kullmann:** None.

Poster

685. Long-Term Potentiation: Kinases and Intracellular Signaling

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Topic: B.08. Synaptic Plasticity

Support: KAKENHI from the MEXT/JSPS

Title: A deficiency of Ca^{2+}-dependent activator protein for secretion 1 affects hippocampal long-term potentiation.

Authors: Y. ISHII[1], C. ISHII[1], Y. SHINODA[1,2], Y. SANO[1], *T. FURUICHI[1];
[1]Tokyo Univ. of Science, Fac. of Sci. and Technol., Noda, Chiba, Japan; [2]Tokyo Univ. of Pharm. and Life Sciences, Envrn. Hlth., Horinouchi, Hachioji, Tokyo, Japan

Abstract: Ca^{2+}-dependent activator protein for secretion 1 (CAPS1) regulates exocytosis of dense-core vesicles and is probably required at the priming step. Recent studies suggested its regulatory role in synaptic transmission by trafficking synaptic vesicles to the active zone. Here we report that CAPS1 also has a critical role in hippocampal plasticity. Because conventional CAPS1 knockout (KO) mice die soon after birth, we utilized forebrain-specific CAPS1 conditional knockout (cKO) mice for this purpose. In the hippocampal CA3-CA1 synapses, the amplitude and paired-pulse facilitation of the synaptic transmission was respectively reduced and increased in CAPS1 cKO. Interestingly, CAPS1 and CAPS2 (another member of the CAPS family) are widely distributed in the hippocampal formation and their major distribution areas are complementary to each other. CAPS2 KO mice is known to show reduced late-phase LTP at CA3-CA1 synapses, whereas CAPS1 cKO mice exhibited an impairment in LTP at CA3-CA1 synapse with an increased post-tetanic potentiation compared with their control littermates. In hippocampal DG-CA3 synapses, the amplitude of the synaptic transmission was respectively reduced. But paired-pulse facilitation was no significant difference, and DG-CA3 synapses are known to cause pre-synaptic LTP. These results suggest that CAPS1 plays a critical role in hippocampal presynaptic plasticity.

Disclosures: Y. Ishii: None. **C. Ishii:** None. **Y. Shinoda:** None. **Y. Sano:** None. **T. Furuichi:** None.

Poster

685. Long-Term Potentiation: Kinases and Intracellular Signaling

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Topic: B.08. Synaptic Plasticity

Support: NIMH Grant MH-38256

Title: A novel structural role for calcium/calmodulin kinase II in hippocampal pyramidal neurons

Authors: *S. INCONTRO[1], C. ASENSIO[2], R. A. NICOLL[1];
[1]UCSF, San Francisco, CA; [2]Dept. of biological sciences, Univ. of Denver, Denver, CO

Abstract: AMPA and NMDA receptors (AMPARs and NMDARs) play a key role in both basal activity and the induction of synaptic plasticity at excitatory synapses. Furthermore the trafficking and the stabilization of these receptors at the postsynaptic density (PSD) modulate synaptic strength. Long-term potentiation (LTP) in the CA1 region of the hippocampus is the primary cellular and molecular model to understanding memory. LTP induction at Schaffer collateral-CA1 synapses requires the activation of postsynaptic NMDA receptors, Ca^{2+} influx through NMDARs, and activation of Ca^{2+}/calmodulin-dependent protein kinase (CaMKII). Within 10s, this activation induces a rapid increase in the number of AMPARs at synapses. CaMKII is necessary for LTP induction and by itself enhances the efficacy of synaptic transmission. Using CRISPR to eliminate CaMKII (α and β isoforms) we have now unveiled an important structural role of CaMKII in maintaining basal synaptic transmission in CA1 pyramidal neurons. We first confirmed that the two isoforms are both important for LTP, since the elimination of both abolished LTP induction. Furthermore basal excitatory postsynaptic currents (AMPAR and NMDAR EPSCs) were strongly reduced. Surprisingly our experiments demonstrate also that CaMKII is needed for basal NMDAR participation at synapses. Molecular replacements with kinase dead forms of CaMKIIα specifically rescued NMDAR transmission uncovering a novel structural role for CaMKII in maintaining NMDARs in the PSD. Together our results uncover important roles for CaMKII in basal synaptic transmission and clarifying its fundamental role in the induction of LTP.

Disclosures: **S. Incontro:** None. **C. Asensio:** None. **R.A. Nicoll:** None.

Poster

685. Long-Term Potentiation: Kinases and Intracellular Signaling

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Topic: B.08. Synaptic Plasticity

Support: NIH T32HL007913

VA Research Service Award Number I01BX000873

Title: Microglia promote post-synaptic depression of sensory relay neurons in the nucleus tractus solitarius (nTS) following acute lung injury in rats

Authors: *D. G. LITVIN[1], C. B. SMITH[1], F. J. JACONO[2];
[1]Physiol. & Biophysics, [2]Dept. of Medicine, Div. of Pulmonary, Critical Care and Sleep Med., Case Western Reserve Univ. Sch. of Med., Cleveland, OH

Abstract: Acute respiratory distress syndrome (ARDS) remains a common cause of respiratory failure, with an estimated annual U.S. mortality rate of over 40%. ARDS patients may experience neurological deficits affecting cognitive and autonomic function, which have been loosely attributed to neuroinflammatory and hypoxic episodes pursuant to the lung injury.
In an intratracheal bleomycin (BM*i*) based model of ARDS, we have previously shown that BM*i* rats displayed elevated brainstem inflammation and synaptic depression in the nTS. We hypothesized that: 1) BM*i* lung injury promotes synaptic depression through changes in post-synaptic glutamate receptor (GluR) expression, and 2) synaptic depression can be ameliorated by minocycline, a microglial/macrophage inhibitor.
7 days following intratracheal saline (S*i*) or BM*i* instillation, BM*i* rats (n=9) showed greater immunoreactivity for the GluR2 subunit of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor compared to control (S*i*) rats (n=8). There were no significant differences in GluR1 and GluR4 levels between groups.
In support of increased post-synaptic insertion of the GluR2 subunit, the amplitudes of spontaneous and tractus solitarii evoked excitatory post-synaptic currents (sEPSCs & TS-eEPSCs) showed reduced inhibition to the non-GluR2 inhibitor 1-Naphthyl acetyl spermine trihydrochloride (NASPM) in BM*i* (n=6) rats compared to S*i* rats (n=7). Moreover, the current rectification index of TS-eEPSCs (recorded in the presence of 0.1μm spermine at +40/-60) was significantly greater in BM*i* (n=4) compared to S*i* (n=4) rats, and the I-V relationship of TS-eEPSCs became more linear in the BM*i* group.
To determine whether microglia/macrophages contributed to the increase in post-synaptic GluR2 in the nTS following BM*i* instillation, BM*i* rats and S*i* rats were treated with intraperitoneal (*ip*) injections of minocycline (25mg/kg) or vehicle for 7 days following lung injury. BM*i* rats treated with minocycline (n=7) exhibited significantly lower GluR2 immunoreactivity in the nTS

compared to vehicle treated BM*i* rats (n=7), but was still significantly greater than *Si* rats treated with vehicle (n=5). Neurons from BM*i* rats treated with saline (n=5) had reduced amplitude and prolonged rise time of sEPSCs that were reversed in minocycline treated BM*i* rats (n=8). Increases in GluR2 expression contribute to post-synaptic depression of sEPSCs in nTS neurons following BM*i* lung-injury, and are reversed by minocycline treatment. We conclude that microglia regulate this process and that neuroinflammation may contribute to dampened autonomic function in the setting of acute lung injury.

Disclosures: D.G. Litvin: None. **C.B. Smith:** None. **F.J. Jacono:** None.

Poster

685. Long-Term Potentiation: Kinases and Intracellular Signaling

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Program#/Poster#: 685.09/G32

Topic: B.08. Synaptic Plasticity

Support: NIH Grant R15NS078645

BYU Graduate Research Fellowship

BYU Mentoring Environment 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[1], M. CHRISTENSEN[1], J. EDWARDS[1];
[1]Brigham Young Univ., Provo, UT; [2]Carnegie Mellon University, Univ. of Pittsburgh, Pittsburgh, PA; [3]UC Irvine, Irvine, CA

Abstract: The hippocampus is thought to mediate learning and memory by altering the strength of synapses within its circuitry. In many cases, this synaptic plasticity can be induced by signaling molecules. Lipid-based signaling molecules called endocannabinoids, can 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. Using patch-clamp electrodes to extract single cells we analyzed the expression of endocannabinoid biosynthetic enzyme mRNA using RT-PCR. In this analysis, we examined cellular expression of several calcium-binding proteins and neuropeptides to determine interneuron subtype. We analyzed cellular expression of several endocannabinoid biosynthetic enzymes, including N-acyl phosphatidylethanolamine phospholipase D (NAPE-

PLD), diacylglycerol lipase alpha, and 12-lipoxygenase, as well as type 1 mGluRs. Preliminary data suggests that stratum oriens interneurons express mRNA necessary for endocannabinoid biosynthetic enzymes. To test the role of endocannabinoids in synaptic plasticity, stratum oriens interneurons were patched and glutamate currents were recorded in the presence of a fatty acid amide hydrolase inhibitor (URB597) to increase endogenous anandamide. We observed a 30% enhancement above baseline (n=7, p<.001) that was blocked by the CB1 inhibitor AM-251 (n=6, p<.001). These results demonstrate a novel endocannabinoid-mediated mechanism for synaptic plasticity in stratum oriens interneurons.

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Poster

685. Long-Term Potentiation: Kinases and Intracellular Signaling

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Topic: B.08. Synaptic Plasticity

Title: ERK2 dependent phosphorylation of the immediate early protein Arc/Arg 3.1

Authors: M. S. ERIKSEN, O. NIKOLAIENKO, *T. KANHEMA, C. R. BRAMHAM; Univ. of Bergen, Bergen, Norway

Abstract: Activity-regulated cytoskeleton-associated protein, Arc, is implicated in multiple forms of synaptic plasticity. It binds to distinct sets of proteins and has functions at synapses and the nucleus. This suggests that Arc function and localization is tightly regulated. Arc transcription, postsynaptic mRNA localization, and translation are all ERK-dependent processes. We suggest that ERK is further implicated in Arc regulation by phosphorylating Arc post-translationally. Activated Erk2 phosphorylates bacterially expressed Arc *in vitro* at five sites, as confirmed by phospho-specific protein staining and subsequent LC-MS/MS analysis. Following LTP induction in the dentate gyrus of the adult rat, phosphorylated Arc was detected using a phospho-site specific antibody to probe immunoprecipitated Arc. We further demonstrate stimulus-evoked, ERK-dependent phosphorylation of Arc in neuroblastoma cell lines. Lentiviral infections of phosphomutated Arc in cultured hippocampal neurons show a time-dependent difference in cytosolic to nuclear expression compared to wildtype. In conclusion, we demonstrate ERK catalyzed phosphorylation of Arc in situ.

Disclosures: M.S. Eriksen: None. **O. Nikolaienko:** None. **T. Kanhema:** None. **C.R. Bramham:** None.

Poster

685. Long-Term Potentiation: Kinases and Intracellular Signaling

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Topic: B.08. Synaptic Plasticity

Support: Wallin Discovery Fund

NIDA T32DA007097

Title: HINT1 protein: a role in spinal neuroplasticity underlying opioid tolerance and neuropathic pain.

Authors: *C. PETERSON[1,2], R. SHAH[3], K. F. KITTO[4], C. FAIRBANKS[5,2,1], C. R. WAGNER[3], G. L. WILCOX[4,6,5];
[1]Pharmaceutics, [2]Exptl. and Clin. Pharmacol., [3]Medicinal Chem., [4]Neurosci., [5]Pharmacol., [6]Dermatol., Univ. of Minnesota, Minneapolis, MN

Abstract: Background: The interactions between the mu-opioid (MOR) and N-methyl-D-aspartate (NMDAR) receptors are an area of intense investigation due to their contribution to maladaptive neuroplasticity. Evidence suggests that their association requires the involvement of histidine triad nucleotide-binding protein (HINT1). Since it is known that spinal blockade of NMDAR prevents the development of opioid analgesic tolerance, we hypothesized that spinal inhibition of HINT1 enzyme may similarly inhibit opioid tolerance. Given the similar mechanisms underlying the development of both opioid analgesic tolerance and neuropathic pain, we reasoned that HINT1 inhibition may reduce the development of neuropathic pain, consistent with observations that antagonism of NMDAR inhibits both developments. Blockade of NMDAR is known to inhibit the development of MOR analgesic tolerance. To address these questions we evaluated HINT1 inhibitors in three models of spinal neuroplasticity: morphine-induced inhibition of NMDA-evoked behavior, endomorphin-2 induced analgesic tolerance, and spared nerve injury.
Experiments: Morphine inhibition of NMDA-induced behavior: Morphine (10 nmol, i.t.) reduced hindlimb-directed scratching and biting behaviors elicited by intrathecal NMDA (0.3 nmol, i.t.). A 5 minute pre-treatment with a HINT1 inhibitor, guanosine-5'-tryptamine carbamate (TpGc), dose-dependently inhibited the morphine-induced reduction of NMDA behavior. Pre-treatment with vehicle alone had no effect.
Endomorphin-2 Induced Analgesic Tolerance: Mice were treated with either TpGc (10 nmol, i.t.) or vehicle. Mice were then injected with endomorphin-2 (10 nmol, i.t.) to induce acute opioid tolerance. At thirty minutes post-injection, the tail flick latencies of mice returned to baseline levels and a probe dose of endomorphin-2 (10 nmol) was administered. Pre-treatment of TpGc prevented the development of endomorphin-2 tolerance.

Spared Nerve Injury: Tactile hypersensitivity was established in mice via partial ligation of the sciatic nerve. TpGc was delivered intrathecally either prior to surgery or one week following surgery, and mice were tested for their von Frey thresholds for several days following surgery. TpGc pre-treatment significantly attenuated the tactile hypersensitivity relative to vehicle-treated controls. This effect persisted through day 5 post-surgery. Additionally, delivery of TpGc one week following induction of neuropathic hyperalgesia transiently reversed tactile hypersensitivity.
Conclusion: These data suggest a role for the HINT1 enzyme in spinal neuroplasticity involving NMDA receptor mechanisms.

Disclosures: C. Peterson: None. **R. Shah:** None. **K.F. Kitto:** None. **C. Fairbanks:** None. **C.R. Wagner:** None. **G.L. Wilcox:** None.

Poster

685. Long-Term Potentiation: Kinases and Intracellular Signaling

Location: Halls B-H

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Program#/Poster#: 685.12/G35

Topic: B.08. Synaptic Plasticity

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Title: High-frequency stimulation induces cholecystokinin release from their terminals that switches long-term potentiation in the auditory cortex

Authors: *W. XIAOYU, X. LI, Y.-T. WONG, J. HE;
City Univ. of Hong Kong, Hong Kong, Hong Kong

Abstract: In our previous studies, we found that the medial temporal lobe influences neocortical plasticity via CCK-positive cortical projection neurons in the entorhinal cortex. In the rat

auditory cortex, long-term potentiation (LTP) could be induced either by high-frequency (HF) stimulation or by only low-frequency stimulation in the presence of CCK. We hypothesized that, with two low-impedance electrodes, LTP of the connection A to B could be induced by stimulating electrode A with HF, or by stimulating electrode B with HF. We then implanted two electrodes in two hemispheres of the auditory cortex, which showed connectivity physiologically. After recording the baseline field potential responses in one hemisphere (electrode B) to the electrical stimulation in the other (electrode A), we applied HF burst stimulation at A. LTP was induced over the above baseline. We further applied HF burst stimulation at B, LTP (with A as the stimulation electrode and B as the recording electrode) was further enhanced. We further hypothesized that HF stimulation was to induce the release of CCK in the stimulation site. In the following experiment, we embedded electrodes and drug-injection needles bilaterally into the rat auditory cortex. After injection of CCK antagonist in the high-frequency stimulation side, rat showed no more LTP, whereas LTP could still be triggered when CCK antagonist was injected on the recording side. The results indicate that HF stimulation induced CCK release from their terminals and CCK strengthened the connection in the surrounding synapses. In conclusion, HF-induced LTP of the direction A to B is produced by the increased input current from A.

Disclosures: **W. Xiaoyu:** None. **X. Li:** None. **Y. Wong:** None. **J. He:** None.

Poster

685. Long-Term Potentiation: Kinases and Intracellular Signaling

Location: Halls B-H

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Topic: B.08. Synaptic Plasticity

Support: Academy of Finland, SA 252302

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University of Helsinki

Sigrid Juselius Foundation

Title: Actin tyrosine-53 phosphorylation in rat hippocampus LTP

Authors: ***J. E. ENGLUND**[1], E. BERTLING[5], R. MINKEVICIENE[5], M. KOSKINEN[6], M. SEGERSTRÅLE[2], E. CASTREN[3], T. TAIRA[4], P. HOTULAINEN[5];
[1]Dept. of Biosci., [2]Dept. of Bisciences, [3]Neurosci. Ctr., [4]Dept. of Vet. Biosci., Univ. of Helsinki,

Helsinki, Finland; [5]Minerva Inst. for Med. Res., Helsinki, Finland; [6]Dept. of Neurosci., Karolinska Institutet, Stockholm, Sweden

Abstract: Our objective was to study the role of actin phosphorylation in dendritic spines during plastic events. Rapid re-organization and stabilization of the actin cytoskeleton in dendritic spines enables cellular processes such as long-term potentiation (LTP). We hypothesized that phosphorylation of actin shortens the filaments, thereby increasing their dynamics (more free – and +-ends).
The common view is that actin binding proteins regulates the stability of actin filaments but in some species, like the slime mold *Dictyostelium,* actin phosphorylation has been shown to be used for this purpose. The tyrosine-53 residue of actin is highly conserved between species and this indicates that this phosphorylation site could have a functional role also in mammals. We show that actin in dendritic spines is dynamically phosphorylated at tyrosine-53 in rat hippocampal neurons.
We did fEPSP (field excitatory post-synaptic potential) recordings, induced LTP in the CA1 of acute rat hippocampal slices and used western blot to study the amount of phosphorylated actin here. The CA1 was frozen 35min after LTP induction and a pY53 (phosphotyrosine-53) specific antibody was used to show a significant increase (2.3 fold) in the amount of phosphorylated actin when compared to control slices. This shows that phosphorylation of actin occurs during LTP, probably to increase the dynamics of the actin filaments and enable the structural changes of the spine head needed for LTP formation.
To study the functional role of actin phosphorylation in LTP we expressed actin constructs modified either to mimic pY53 actin (Y53E, Tyr replaced with Glu) or to be unsuitable for phosphorylation (Y53A, Tyr replaced with Ala) and for control we used wildtype actin (wt). The constructs were conjugated with GFP to enable detection of expression. We used in vivo lenti-virus injections (under isoflurane anesthesia) during P1-4 (postnatal day) to perform transduction of the constructs. After P14 we did fEPSP recordings in CA1 and compared the size of LTP in slices expressing the different actins. Expression of the pY53 actin mimicking actin reduced the size of LTP as compared with wt and Y52A expression (22±4%, n=11 vs. 37±4%, n=22).
The conclusion is that actin is phosphorylated at tyrosine 53 during expression of LTP and that this mechanism is obstructed by the expression of pY53 mimicking actin. The idea is that if many actin filaments already are very short and dynamic, due to Y53E expression, there is a reduced number of filaments that can increase their dynamics and re-arrange as a response to the LTP induction protocol. What kinases/phosphatases that are involved in regulating actin phosphorylation is still unknown.

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Poster

685. Long-Term Potentiation: Kinases and Intracellular Signaling

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Support: This work was supported by National Science Center grant SONATA/2014/13/D/NZ4/03045.

Title: The temporal crosstalk of NMDARs and matrix metalloproteases activity determines the magnitude of EPSP-to-spike potentiation and c-Fos expression in the CA1 hippocampal region

Authors: P. BRZDAK[1,2], J. W. MOZRZYMAS[1,2], *T. WOJTOWICZ[1];
[1]Lab.Neuroscience, Wroclaw Med. Univ., Wroclaw, Poland; [2]Lab. of Cell. Neurobiology, Dept. of Animal Mol. Physiol., Wroclaw Univ., Wroclaw, Poland

Abstract: Matrix metalloproteases (MMP) activity supports reorganization of extracellular matrix, synaptic plasticity, learning and memory. However, the functional and molecular mechanisms remain largely unknown. Recently, MMP-3 was shown to cleave NR1 subunit of NMDARs and was upregulated following hippocampus-dependent learning. NMDARs are calcium permeable channels that are necessary for induction of long-term EPSP-to-spike (E-S) potentiation and synthesis of pro-plasticity proteins via upregulation of gene expression. We hypothesized that MMP-3 activity might affect NMDAR function and downstream signaling cascades involved in neuronal plasticity but this issue has not been addressed before. We thus started with description of the temporal requirement for MMP-3 and NMDARs activity on the magnitude of E-S plasticity in CA1 hippocampal region in acute brain slices of C57BL6 adult mice. We found that E-S potentiation following high frequency stimulation (HFS, 4x100Hz, field-potentials technique) was significantly reduced when NMDAR antagonist APV (50μM) or MMP-3 inhibitor NNGH (10μM) was applied before or up to 15-30 minutes post HFS. We next analyzed immediate early gene product cFos protein expression in fixed sections of recorded slices. We found that NNGH and APV effects on E-S potentiation were temporally matched with expression of immediate early gene product cFos in CA1 pyramidal neurons. Next we asked whether exogenous recombinant MMP-3 alters NMDARs function in two models: NMDAR-mediated local field potentials evoked with glutamate puffs in acute brain slices (glutamate and D-serine in the presence of DNQX and nifedipine) and NMDA-evoked Ca^{2+} waves in cultured hippocampal neurons (measured with Fura2 Ca^{2+} indicator). We found that exogenous MMP-3 enhanced somatodendritic Ca^{2+} waves following multiple exposure to NMDA (15-20 min) in vitro and dendritic glutamate-evoked NMDAR-mediated field potentials. In conclusion, MMP-3 may modulate NMDARs function, postsynaptic Ca^{2+} entry and gene expression but this process

is time-locked vs. episode of enhanced neuronal activity. Our study also provides a new insight into mechanism of MMP-dependent hippocampal neuronal plasticity, learning and memory.

Disclosures: **P. Brzdak:** None. **J.W. Mozrzymas:** None. **T. Wojtowicz:** None.

Poster

685. Long-Term Potentiation: Kinases and Intracellular Signaling

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Topic: B.08. Synaptic Plasticity

Support: Wellcome-DBT India Alliance Intermediate Fellowship (awarded to Dr. Suhita Nadkarni)

Title: Post-synaptic calcium signaling and plasticity: role of intracellular stores

Authors: ***G. MAHAJAN**, S. NADKARNI;
Biol., IISER, Pune, Pune, India

Abstract: A transient rise in calcium in the postsynaptic dendritic spine is a necessary trigger for activity-dependent synaptic potentiation/depression seen in a variety of stimulation protocols. Models for NMDAR-dependent long-term potentiation (LTP), including those for spike-timing-dependent plasticity (STDP), have traditionally attempted to link the direction and magnitude of synaptic change to the peak of the calcium elevation, or, more generally, to the time course of the calcium signal (calcium control hypothesis). In these models, NMDA-gated and voltage-gated channels provide the only sources of calcium entry into the cell. However, calcium stores in the sarco-endoplasmic reticulum (SER) can also potentially contribute to the calcium dynamics in dendrites and spines. We study how the release of calcium from intracellular stores via multiple pathways can modulate the calcium signal elicited by pre and post synaptic activation in different scenarios that span a broad range of timescales. There is growing experimental evidence implicating a role for SER calcium channels such as those coupled to ryanodine and IP3 receptors in regulation of diverse forms of plasticity. Using a computational model of a hippocampal neuron that incorporates considerable level of biophysical detail, we attempt to quantify the influence that presence of the ER machinery can have on dendritic membrane and calcium dynamics, and in turn, on the downstream signalling networks involved in LTP/LTD induction. Such an understanding will contribute to a more complete mechanistic picture of spine maintenance and plasticity. Our work should also help to better characterize how familial Alzheimer's disease-linked mutations in presenilin, a protein known to regulate calcium release from the ER, could disrupt synaptic function on the post-synaptic side.

Disclosures: **G. Mahajan:** None. **S. Nadkarni:** None.

Poster

685. Long-Term Potentiation: Kinases and Intracellular Signaling

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Topic: B.08. Synaptic Plasticity

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Title: GRASP1 regulates synaptic plasticity and learning through endosomal recycling of AMPA receptors

Authors: ***S.-L. CHIU**[1,2], G. H. DIERING[1,2], B. YE[3], K. TAKAMIYA[4], C.-M. CHEN[5], Y. JIANG[6], N. TEJAS[6], C. E. SCHWARTZ[7], T. WANG[6], R. L. HUGANIR[1,2];
[1]Solomon H. Snyder Dept. of Neurosci., Johns Hopkins Univ. Sch. of Med., Baltimore, MD; [2]Kavli Neurosci. Discovery Inst., Baltimore, MD; [3]Univ. of Michigan, Ann Arbor, MI; [4]Univ. of Miyazaki, Miyazaki, Japan; [6]Inst. of Genet. Med., [5]Johns Hopkins Univ., Baltimore, MD; [7]Greenwood Genet. Ctr., Greenwood, SC

Abstract: Learning depends on experience-dependent modification of synaptic efficacy and neuronal connectivity in the brain. Recycling endosomes play an essential role in regulating activity-dependent synaptic strengthening and spine morphogenesis at glutamatergic synapses *in vitro,* but the underlying molecular mechanisms involved and their role *in vivo* remains unclear. Here we provide direct evidence for physiological roles of the recycling endosome protein GRASP1 in glutamatergic synapse function and behavior. Mice lacking GRASP1 showed abnormal excitatory synapse number and synaptic plasticity. Moreover, learning-induced synaptic AMPA receptor incorporation as well as multiple forms of hippocampal-dependent learning and memory were impaired in these mice. We found that GRASP1 interacted with AMPA receptors and was recruited to synapses in an activity-dependent manner in neurons,

which may underlie experience-induced synaptic AMPA receptor delivery and synaptic strengthening necessary for subsequent learning and memory. Interestingly, we identified two GRASP1 point mutations from patients with severe intellectual disability. These GRASP1 mutations displayed altered binding to recycling endosomal proteins and impaired activity-dependent AMPA receptor recycling, suggesting a potential role for GRASP1 in the pathophysiology of human cognitive disorders.

Disclosures: **S. Chiu:** None. **G.H. Diering:** None. **B. Ye:** None. **K. Takamiya:** None. **C. Chen:** None. **Y. Jiang:** None. **N. Tejas:** None. **C.E. Schwartz:** None. **T. Wang:** None. **R.L. Huganir:** None.

Poster

685. Long-Term Potentiation: Kinases and Intracellular Signaling

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Topic: B.08. Synaptic Plasticity

Support: NSERC Grant

Title: Differential roles of L-type calcium channel and NMDA receptor in early odor preference learning in mice

Authors: ***B. MUKHERJEE**[1], C. HARLEY[2], Q. YUAN[3];
[1]MEDICINE, MEMORIAL UNIVERSITY OF NEWFOUNDLAND, St John's, NL, Canada;
[2]Psychology, [3]Medicine-Biomedical Sci., Mem. Univ. of Newfoundland, St. John's, NL, Canada

Abstract: Calcium as a 2nd messenger is critical in transmitting synaptic signals to the nucleus of the neuron to initiate gene transcription required for long-term memory. Voltage-gated calcium channels such as L-type calcium channels (LTCC) and NMDA receptors (NMDARs) serve as the principle sites for calcium entry on the membrane. NMDARs permit calcium entry at the synaptic site upon coincident presynaptic activity and postsynaptic depolarization, while LTCCs have an important role in translating cytosolic calcium increases to nucleus gene expression. Using early odor preference learning in mice as a model system, we study how LTCCs and NMDARs interact to promote memory formation. Early odor preference learning is induced by pairing a novel odor with a tactile stimulation (e.g. stroking a pup with a brush). We focus on the anterior piriform cortex (aPC) which has been previously identified as a primary site for early odor preference learning. Using calcium imaging in aPC slices of postnatal 8-10 mice, we show that LTCC activation is dependent on NMDAR activation. Either D-APV (NMDAR antagonist) or nifedipine (LTCC antagonist) reduced somatic calcium transients in pyramidal cells evoked

by lateral olfactory tract (LOT) stimulation. However, nifedipine application did not result in further reduction of calcium in the presence of D-APV, while activating LTCCs directly with BayK-8644 enhanced LOT evoked calcium transients in D-APV. We next show that long-term potentiation of pyramidal cell calcium transients is dependent on both NMDARs and LTCCs. Blocking either NMDARs or LTCCs prevented the long-term calcium increase that could be otherwise induced in normal aCSF. We then studied the roles of these two channels in mediating early odor preference learning and AMPA receptor (AMPAR) trafficking to the synaptic membrane. Blocking NMDARs in the aPC prevented short-term (3h) and long-term (24h) odor preference memory, and both memories were rescued when BayK-8644 was co-infused with D-APV. Blocking LTCCs only prevented 24h memory and spared 3h memory. Synaptic AMPAR expression at 3h was elevated in the vehicle infused learning group, as well as the nifedipine group and the D-APV+BayK-8644 group. However, it was no longer elevated in the nifedipine group at 24h. The patterns of AMPAR membrane expression mirror behavioral outputs at both 3h and 24h, suggesting AMPAR insertion is critical for both 3h and 24h memories. These results suggest LTCC is not necessary for short-term odor preference memory which has been shown to be protein-synthesis independent. Further experiments will focus on the input-specificity of odor preference memory induced by NMDAR and LTCC activation.

Disclosures: **B. Mukherjee:** None. **C. Harley:** None. **Q. Yuan:** None.

Poster

685. Long-Term Potentiation: Kinases and Intracellular Signaling

Location: Halls B-H

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Topic: B.08. Synaptic Plasticity

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Title: High fat diet impairs synaptic plasticity by palmitoylation and reduces trafficking of AMPA receptor GluR1 subunit

Authors: ***M. SPINELLI**, S. FUSCO, M. MAINARDI, F. SCALA, A. MATTERA, M. D'ASCENZO, C. RIPOLI, D. D. LI PUMA, C. GRASSI;
Human Physiol., Catholic Univ. of the Sacred Heart, Roma, Italy

Abstract: High-fat diet (HFD) causes metabolic alterations including dyslipidemia and insulin resistance, and impacts on hippocampal synaptic transmission and plasticity through still poorly understood molecular mechanisms. In hippocampi of mice fed with HFD for 6 weeks we found increased levels of palmitic acid and reduced long-term potentiation (LTP) at CA3-CA1 synapses (SD= 186.2 ± 19.9, HFD= 123.6 ± 8.1; n=9 for each group; $p<0.05$). Palmitic acid is the substrate for a post-translational modification called palmitoylation, that regulates trafficking and signaling pathways of many proteins involved in synaptic function. To test whether the effect of HFD on synaptic function is mediated by an altered palmitoylation of synaptic proteins, we analyzed the palmitoylation of both NMDA and AMPA receptor (AMPAR) subunits in the hippocampus of HFD-fed mice. HFD specifically increased the palmitoylation of AMPAR subunit GluR1 (+30% ± 1; n=5; $p<0.05$), that is the main effector of LTP, and reduced its phosphorylation at serine 845 (pGluR1-S845) (-35% ± 1; n=4; $p<0.05$). To deep investigate the molecular mechanism underlying the diet-dependent change of GluR1 palmitoylation we performed an *in vitro* model resembling the *in vivo* metabolic stress. Mouse hippocampal neurons treated for 24h with (20 nM) insulin and (200 μM) palmitic acid (IPA) showed: i) an alteration of GluR1 palmitoylation/S845 phosphorylation balance; ii) reduced GluR1 trafficking to the plasma membrane and lower binding with PSD-95; iii) the loss of GluR1 activation upon a chemical LTP protocol. Accordingly, autaptic hippocampal neurons exposed to IPA showed a significant reduction in amplitude of AMPAR evoked post-synaptic currents (-45% ± 1 of controls; n=20; $p<0.001$). Moreover, GluR1/PSD-95 interaction was inhibited by IPA treatment (-70% ± 2 of controls; n=4; $p<0.01$). Finally, a complete rescue of both GluR1 palmitoylation and LTP impairment was observed in organotypic brain slices exposed to the competitive inhibitor of palmitic acid, 2-bromo palmitate (5 μM for 24 h). Collectively, our data indicate that IPA affects synaptic plasticity altering both palmitoylation and trafficking of GluR1. Our findings reveal a novel molecular mechanism underlying the diet-related synaptic dysfunction.

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Poster

685. Long-Term Potentiation: Kinases and Intracellular Signaling

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Topic: B.08. Synaptic Plasticity

Support: NIH P50MH086403

Title: Conditional deletion of LRRTM proteins impairs long term potentiation (LTP) in hippocampal CA1 pyramidal neurons in adult mice

Authors: *M. BHOURI[1], T. J. SIDDIQUI[2,3], P. TEMKIN[1], W. MORISHITA[1], D. GOSWAMI[1], S. BOTELHO[4], T. C. SUDHOF[4], A. CRAIG[5], R. C. MALENKA[1];
[1]psychiatry and behavioral sciences, Stanford Univ., Stanford, CA; [2]Dept. of Physiol. and Pathophysiology, Univ. of Manitoba, Manitoba, MB, Canada; [3]Kleysen Inst. for Advanced Medicine, Hlth. Sci. Ctr., Winnipeg, MB, Canada; [4]Dept. of Mol. and Cell. Physiol., Howard Hughes Institute, Stanford Univ., Stanford, CA; [5]Brain Res. Ctr. and Dept. of Psychiatry of British Columbia, Vancouver, BC, Canada

Abstract: Leucine-rich repeat transmembrane (LRRTM) proteins are postsynaptic cell adhesion molecules. Like neuroligins, LRRTM proteins bind to presynaptic neurexins and form transynaptic adhesion complexes. LRRTM proteins regulate synapse formation *in vitro* and appear to influence synaptic function *in vivo*. Indeed, double knockdown (DKD) of LRRTM 1 and 2 strongly impairs LTP at both neonatal and mature Schaffer collateral-CA1 (SC-CA1) synapses but their detailed role in synaptic plasticity remains unclear.
Here we used viral-mediated knockout (KO) of LRRTM proteins *in vivo* to examine their role in long-term potentiation (LTP) and basal synaptic transmission. We performed stereotaxic injections of lentivirus expressing Cre recombinase fused to GFP in floxed LRRTM1 and floxed LRRTM2 conditional knockout (KO) mice at P21 to delete LRRTM 1 alone, LRRTM 2 alone or both LRRTM1 and 2 in CA1 pyramidal neurons. Acute hippocampal slices from these animals were prepared 10-15 days later and whole-cell recordings from CA1 pyramidal neurons were made to record excitatory postsynaptic currents (EPSCs) evoked by electrical stimulation of SCs. We find that LTP is strongly impaired in LRRTM 1 KO, LRRTM 2 KO and in LRRTM 1-2 double KO (DKO) CA1 neurons. Overexpression of LRRTM 2 alone is sufficient to restore LTP in LRRTM 2 KO and LRRTM 1/2 DKO CA1 neurons. Assays of basal synaptic transmission (i.e. AMPAR/NMDAR ratios, paired-pulse ratios, miniature EPSCs) revealed that LRRTM1/2 KO had no detectable effects on basal synaptic responses. Molecular replacement experiments using mutant forms of recombinant LRRTM2 are on-going to test the hypothesis that LRRTMs are required for the maintenance of the increased number of synaptic AMPA receptors following the induction of LTP.

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Poster

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Topic: B.08. Synaptic Plasticity

Title: Dehydration lowers the threshold for CREB phosphorylation in mouse hippocampus

Authors: A. VASHISHT[1], M. MORYKWAS[1], *A. N. HEGDE[2], L. ARGENTA[1], M. MCGEE[1]; [1]Wake Forest Univ. Sch. of Med., Winston Salem, NC; [2]Dept. of Biol. & Env. Sci., Georgia Col. and State Uni, Milledgeville, GA

Abstract: Local dehydration ex-vivo was previously shown in mouse hippocampal slices to impair synaptic plasticity by preventing late-phase long-term potentiation (L-LTP) induction. There was also an increase in basal synaptic transmission upon dehydration indicating hyperexcitability of neurons. In addition, during subthreshold stimulation of L-LTP, the levels of phosphorylated cAMP response element-binding protein (pCREB) increased upon proteasome inhibition by β-lactone. Here, we examined the effect of dehydration on modulation of CREB phosphorylation by subthreshold stimulation. Controlled dehydration was achieved with inert non penetrating polymers either PEG (Polyethylene glycol-8000) or Dextran-10,000 dissolved in artificial cerebrospinal fluid (ACSF) to attain colloidosmotic pressures of 0 mmHg in control ACSF and 54 mmHg, 101 mmHg, and 196 mmHg with PEG and 109 mmHg with Dextran. This pressure range amounts to <5% of plasma osmotic pressure (~5800 mmHg).
Electrophysiological recordings were performed at 32°C. Basal synaptic responses were measured upon incremental stimuli (10-35V) at each bath pressure. To investigate the effect of dehydration on CREB phosphorylation, hippocampal slices were equilibrated at a colloid osmotic pressure of 196 mmHg for 30 min. Slices in ACSF were used as control. Thereafter, subthreshold stimulation for L-LTP induction was given as two 100-Hz trains spaced 5 min apart. Slices were fixed within 1 min, stained with anti-pCREB (Ser-133) antibody and quantified in confocal images using Image J software. For proteasome inhibition, slices were treated with β-lactone (25 μM) in both control and dehydrated slices. Experiments were reproduced in at least 6 mice.
Basal synaptic transmission increased in proportion to dehydration and the effect was reversible and independent of the polymer used to control hydration. After subthreshold stimulation, CREB phosphorylation increased in dehydrated slices but not in control slices. Upon proteasome inhibition, pCREB levels increased in both non-dehydrated and dehydrated slices to similar levels. These results indicate that dehydration, like proteasome inhibition, also increases CREB phosphorylation. This increase may be related to the observed hyperexcitability of neurons and the consequent impairment of synaptic plasticity.

Disclosures: **A. Vashisht:** None. **M. Morykwas:** None. **A.N. Hegde:** None. **L. Argenta:** None. **M. McGee:** None.

Poster

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Topic: B.08. Synaptic Plasticity

Support: CIHR Grant MOP-130526

Title: Cadherins mediate cocaine-induced synaptic plasticity and behavioral conditioning.

Authors: ***A. K. GLOBA**[1], F. MILLS[1], S. LIU[3], C. M. COWAN[1], A. G. PHILLIPS[2], S. L. BORGLAND[3], S. X. BAMJI[1];
[1]Cell. & Physiological Sci., [2]Psychiatry, Univ. of British Columbia, Vancouver, BC, Canada; [3]Dept. of Physiol. and Pharmacol., Univ. of Calgary, Calgary, AB, Canada

Abstract: Variations in cadherin-catenin adhesion complex genes are associated with multivariate drug use, however the role of these proteins in drug-mediated synaptic plasticity has not been defined. Here we demonstrate that classical cadherins N-cadherin, R-cadherin, and cadherins -7, -8, and -11 are expressed in dopaminergic (DA) and non-dopaminergic neurons of the VTA and that N-cadherin is localized to both excitatory and inhibitory synapses being formed onto these DA neurons. Inhibiting intercellular N-cadherin interactions abolished spike-timing dependent plasticity in the VTA, indicating that cadherins are important mediators of synapse plasticity in this region. Using immuno electron microscopy we demonstrate that following cocaine-mediated conditioned place preference (CPP), cadherin and GluA1 are significantly recruited to the synaptic membrane of excitatory synapses being formed onto dopaminergic neurons. This is reversed following extinction of CPP. Moreover, stabilizing cadherin at the membrane using a transgenic mouse model significantly attenuates cocaine-mediated CPP and spike-timing dependent plasticity. These results show that cadherins play an important role in synaptic plasticity in the VTA and may be involved in structural changes at synapses caused by cocaine use.

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Poster

685. Long-Term Potentiation: Kinases and Intracellular Signaling

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 685.22/G45

Topic: B.08. Synaptic Plasticity

Support: ONR Global N62909-15-1-2002

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Title: Molecular mechanisms underlying the effects of unilateral anodal transcranial direct current stimulation (tDCS) on synaptic function in the mouse hippocampus

Authors: *M. V. PODDA[1], S. COCCO[1], G. LIVRIZZI[1], S. A. BARBATI[1], C. COLUSSI[2], S. FUSCO[1], L. LEONE[1], C. RIPOLI[1], C. GRASSI[1];
[1]Inst. of Human Physiology, Univ. Cattolica, Rome, Italy; [2]Inst. of Cell Biol and Neurobiol, CNR, Rome, Italy

Abstract: Transcranial direct current stimulation (tDCS) has been reported to enhance cognitive and motor performances in healthy subjects as well as in patients suffering from neuropsychiatric diseases. As such the molecular mechanisms underlying tDCS effects are being increasingly investigated. We recently demonstrated that a single 20-min anodal tDCS session induced long-lasting enhancement of long-term potentiation (LTP) at hippocampal CA3-CA1 synapses and improved hippocampal-dependent spatial and working memory through a mechanism involving epigenetic regulation of Bdnf gene expression (Podda et al., 2016). Here we further investigated the early molecular events triggered by anodal tDCS leading to enhanced synaptic plasticity in the hippocampus. To this aim we evaluated the involvement of Ca^{2+}-activated pathways given that increased intracellular Ca^{2+} levels likely occur following anodal tDCS-induced neuronal depolarization. We found that 3 h after stimulation, hippocampi from tDCS-mice showed increased CaMKII phosphorylation at Thr286 compared to sham-stimulated controls, as revealed by Western immunoblot analyses (+102% *vs.* controls; $P<0.05$). Additionally, immunoprecipitation assays revealed that S-nitrosylation of the histone deacetylase 2 (HDAC2) was increased in tDCS-mice compared to controls (+450%; $P<0.05$), suggesting that HDAC2 inhibition contributes to the enhanced acetylation of the Bdnf promoter we observed following tDCS. These data suggest that transient increases in intracellular Ca^{2+} levels by tDCS initiate molecular cascades leading to long-lasting chromatin remodeling of Bdnf gene. We also further investigated the molecular pathways downstream of Bdnf/TrkB signaling activated by tDCS,

focusing on glycogen synthase kinase-3β (GSK-3β). We found that anodal tDCS enhanced GSK-3β phosphorylation at Ser-9 (+155%; $P<0.005$) that inhibits the enzyme activity. Interestingly, reduced GSK-3β activity is known to enhance LTP and modulate neuronal excitability in the hippocampus. Of note, patch-clamp recordings showed that the number of action potentials elicited by depolarizing current injection (150 pA) was significantly increased in hippocampal slices obtained from tDCS-mice compared to control slices (10.4 ± 1.1 *vs.* 6.8 ± 1.0 in controls; $P<0.05$). Collectively, our findings provide further insights into the mechanisms underlying tDCS effects on synaptic function, that ground the use of tDCS for improving brain functions under physiological and pathological conditions.

Disclosures: **M.V. Podda:** None. **S. Cocco:** None. **G. Livrizzi:** None. **S.A. Barbati:** None. **C. Colussi:** None. **S. Fusco:** None. **L. Leone:** None. **C. Ripoli:** None. **C. Grassi:** None.

Poster

685. Long-Term Potentiation: Kinases and Intracellular Signaling

Location: Halls B-H

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Topic: B.08. Synaptic Plasticity

Support: Trinity College Faculty Research Committee

Title: Chronic caffeine exposure disrupts hippocampal LTP induction but not its duration in freely behaving rats.

Authors: *J. H. BLAISE[1], J. E. PARK[2], N. J. BELLAS[3], T. M. GITCHELL[4], V. PHAN[4]; [1]Engin., [2]Biol., [3]Chem., [4]Neurosci., Trinity Col., Hartford, CT

Abstract: Caffeine is one of the most widely consumed psychoactive stimulants in the world. Its effects on neurological functions, such as increases in alertness and improvements in motor skills, have promoted its use throughout history. Although there have been many studies of the short-term cognitive benefits of caffeine intake, its long term effects have been widely debated. Despite this, it is estimated that nearly 60% of American adults consume caffeine on a daily basis, resulting in annual spending of $40 billion. In this study we examined the effects of chronic caffeine exposure on long term potentiation, a cellular model of learning and memory. Caffeine water (1.0g/L) was administered to 10-17 week-old rats at least three weeks prior to experimentation, after which these rats underwent stereotaxic surgery to chronically implant a stimulating electrode in the perforant path—a major hippocampal multimodal sensory input pathway—and a recording electrode in the dentate gyrus—the first branch of the hippocampal tri-synaptic circuit. This allowed for reliable evoked field potentials to be recorded in the dentate

gyrus and quantified by computer analysis. Following a one-week postsurgical recovery period, LTP was induced in the dentate gyrus using a standard burst tetanization protocol (100-pulse, 5-Hz theta-burst stimulation, TBS) applied to the perforant path. All experimental protocols were performed according to the United States Public Health Service's Guide for the Care and Use of Laboratory Animals and were approved by Trinity College Institutional Animal Care & Use Committee. After at least 5-7 days of post-surgical recovery, population spike amplitude measures of LTP induction were acquired in freely behaving rats using electrophysiological recording techniques. Our results indicate that rats that were exposed to caffeine (n =14) show a statistically significant ($p < 0.05$) lower level of LTP induction compared to controls (n =15). However, no statistically significant differences were found in the duration or persistence of LTP, suggesting that caffeine disrupts LTP induction but not its duration. More studies are needed to determine the exact mechanism through which caffeine alters LTP induction.

Disclosures: **J.H. Blaise:** None. **J.E. Park:** None. **N.J. Bellas:** None. **T.M. Gitchell:** None. **V. Phan:** None.

Poster

685. Long-Term Potentiation: Kinases and Intracellular Signaling

Location: Halls B-H

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Program#/Poster#: 685.24/G47

Topic: B.08. Synaptic Plasticity

Support: IIT Madras (SC,MS)

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Title: A computational model of astrocyte induced modulation of synaptic plasticity and normalization

Authors: O. V. SREERAG[1], R. PHILLIPS[1], S. CHAKRAVARTHY[1], *M. SUR[2];
[1]IIT Madras, Chennai, India; [2]Dept. of Brain and Cognitive Sci., Picower Inst. for Learning and Memory, MIT, Cambridge, MA

Abstract: It has been suggested that perisynaptic astrocytic processes have a role in synaptic plasticity. Here we propose a simple model for astrocytic modulation of synaptic plasticity. The model consists of presynaptic, postsynaptic and astrocyte compartments of the tripartite synapse. Presynaptic activation initiates neurotransmitter release into the synaptic cleft. This neurotransmitter binds with NMDA receptors leading to calcium influx into the postsynaptic compartment. However, NMDAR opening is also contingent upon postsynaptic potential-

dependent magnesium blockage. The postsynaptic compartment is influenced by both the presynaptic compartment and astrocytic processes. Astrocytes release gliotransmitters including D-serine and glutamate, which in turn regulate the synaptic neurotransmitter concentration and influence the postsynaptic calcium concentration. Following the 'calcium control hypothesis,' we assume that the postsynaptic calcium controls the "synaptic strength", w, which corresponds to the strength of response of AMPA receptors to synaptic glutamate. This synaptic strength is modelled by a cubic nonlinearity exhibiting bistable dynamics.
The proposed model is comprised of a presynaptic variable (firing rate) and postsynaptic voltage (controlled by an external current), which are independently varied to display LTD and LTP. Interestingly, the model exhibits BCM-like dynamics wherein the plasticity switches from LTD to LTP at a threshold value of postsynaptic voltage. A key feature of the model is that this threshold voltage is modulated by gliotransmitter released by the astrocyte. We next simulate weight dynamics at two synapses supplied by the same astrocyte such that that the total gliotransmitter released to the two synapses is conserved. Simulations showed a mechanism of normalization of the two synaptic weights in that growth in one synapses is accompanied by attenuation in the other.
In summary, the model demonstrates: (1) astrocyte induced modulation in LTD to LTP threshold, and (2) weight normalization of multiple synapses controlled via astrocytic gliotransmitter release.

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Poster

686. Mechanisms of Synaptic and Neuronal Plasticity

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 686.01/G48

Topic: B.08. Synaptic Plasticity

Support: CIHR

NSERC

Title: Unveiling neuronal differences in chloride extrusion capacity

Authors: F. FERRINI[1], L.-E. LORENZO[2], M. COTTET[2], A. GODIN[3], N. DOYON[2], *Y. DE KONINCK[4];
[1]Univ. or Turin, Turin, Italy; [2]Laval Univ., Quebec, QC, Canada; [3]Univ. of Bordeaux, Bordeaux, France; [4]Cell. & Mol. Neurobio., Laval Univ. / IUSMQ, Quebec, QC, Canada

Abstract: The strength of synaptic inhibition in the adult neurons relies on their capacity to maintain intracellular chloride (Cl^-) concentration, and the main regulator of intracellular Cl^- concentration is the K^+-Cl^- co-transporter 2 (KCC2). As KCC2 function is not homogenous across the CNS, these differences may significantly affect the integration of inhibitory inputs. Here we combined different approaches to explore the impact of variable Cl^- extrusion capacities in nociceptive neurons of the superficial spinal dorsal horn, which includes projections neurons in lamina I and interneurons in lamina II. By imposing a Cl^- load, E_{GABA} recorded in lamina I was more depolarized than in lamina II, indicating a weaker Cl^- extrusion capacity in the former. Notably, we did not observe any difference when E_{GABA} was measured under low chloride conditions (including by gramicidin-perforate patch clamp), suggesting that a Cl^- load is required to detect apparently subtle differences. These data were replicated by performing Cl^- imaging in MQAE-loaded spinal cord slices. Indeed, when synaptic activity was completely blocked, no interlaminar differences in Cl^- concentration were detected in dorsal horn, conversely in presence of normal synaptic activity a clear gradient in Cl^- concentration was observed. The gradient was further increased by enhancing synaptic activity with capsaicin. We confirmed that the lower Cl^- extrusion capacity in lamina I neurons affects synaptic inhibition in an activity dependent manner by stimulating high frequency inhibitory activity. These experiments showed that under sustained inhibitory input, lamina I neurons accumulate Cl^- faster than lamina II. Functional experiments were mirrored by immunohistochemical analysis of KCC2 expression which confirmed a lower level of KCC2 in lamina I under the control of TrkB receptor signaling. Following nerve injury, KCC2 expression strongly decreased in both laminae, but still KCC2 in lamina I was the lowest. Our computer simulation indicated that such a low KCC2 level in lamina I of nerve injured rats fails to maintain Cl^- gradient when synaptic activity increases. Thus, constitutive differences of KCC2 activity has a strong impact on the efficiency of enhanced inhibition under both normal and pathological settings.

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Poster

686. Mechanisms of Synaptic and Neuronal Plasticity

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 686.02/G49

Topic: B.08. Synaptic Plasticity

Title: Vanilline protects the hippocampal neuronal function from age-related deterioration

Authors: *K. ITO, S. TANIGUCHI, M. HANAFUSA, H. TSUBONE, D. YAMANAKA, M. KUWAHARA;
Univ. Tokyo, Tokyo, Japan

Abstract: Vanillin (4-hydroxy-3-methoxybenzaldehyde) belongs to the vanilloids and is widely used as an additive in food or cosmetic as a constituent of vanilla flavor. Recently, vanillin is reported to play a role in antioxidative events and antiaging. However, the effect of vanillin on age-related synaptic dysfunction is unknown so far. In this study, we investigated the chronic and acute effect of vanillin on the hippocampal Schaffer collateral - CA1 synapses using old C57BL/6J (52-weeks old). We randomly assigned the mice for the vanillin-supplemented group and the control group, and allowed them to access freely to tap water for control group and tap water including 0.17% vanillin for vanillin-supplemented group for 4 weeks, respectively. First, we recorded the field excitatory postsynaptic potential (fEPSP) slope of the vanillin-supplemented mice. The slopes of fEPSPs of vanillin-supplemented group were significantly larger than those of control group. However, we could not find the difference in the paired-pulse ratio, the representative of the short-term plasticity, between these groups. Then, we checked the long-term synaptic plasticity by determining the long-term potentiation (LTP). The degree of LTP in vanillin-supplemented group was comparable with the control group. We tested the effect of vanillin supplementation with behavioral test. Morris water maze test and Y-maze test showed that there were no significant differences in spatial memory between these groups. Next, we tested the acute effect of vanillin for the synaptic transmission and plasticity as vanillin is reported to cross the blood-brain barrier. Bath application of vanillin (1 mM) increased the slope of fEPSPs. In the same way, the degree of LTP increased under vanillin perfusion. Moreover, we conducted the biological antioxidant potential (BAP) assay for evaluation of reduction capacity which restores the oxidative level elevated in old animals. However, the BAP level in vanillin-supplemented group was comparable with that of control group. These results indicate that vanillin supplementation does not restore the age-related impairment of recognition or synaptic plasticity but it augments the synaptic transmission independently of reduction capacity. Our data about vanillin in this study is expected for improvement of neuronal function in the old animals in which the synaptic dysfunction progresses age-dependently.

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Poster

686. Mechanisms of Synaptic and Neuronal Plasticity

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 686.03/G50

Topic: B.08. Synaptic Plasticity

Title: Acetyl-L-carnitine improves age-dependent impairment of LTP of the senescence-accelerated mouse prone 8

Authors: *S. TANIGUCHI, H. TAKIMOTO, M. HANAFUSA, H. TSUBONE, D. YAMANAKA, M. KUWAHARA, K. ITO;
Univ. of Tokyo, Tokyo, Japan

Abstract: Acetyl-L-carnitine (ALCAR) is an acetyl moiety of carnitine. The concentration of ALCAR and carnitine declines with age in skeletal muscle, cerebral cortex and hippocampus. Recently, it is reported that external addition of ALCAR improves cognitive impairment in old animals. However, little is known about the effect of ALCAR on synaptic functions in aged animals. In this study, we investigated the effect of ALCAR on the hippocampal CA1 synapses using an animal model of aging called senescence-accelerated mouse prone 8 (SAMP8) which shows not only short life-span or rapid advancement of senescence but also age-related impairments of learning and memory. We obtained the hippocampal slices from the mice and evaluated the synaptic function by filed recordings. High-frequency stimulation (HFS) evoked long-term potentiation (LTP), which is a well-established form of synaptic plasticity, in the hippocampal synapses of 6 month-old SAMR1, whereas HFS induced smaller degree of LTP in the age-matched SAMP8 synapses. However, supplementation of ALCAR (0.5 g/kg/day) for 6 weeks improved the degree of LTP in 6 month-old SAMP8 but not in SAMR1. As ALCAR is known to cross the blood-brain barrier and directly impinge on synapses, we examined the acute effect of ALCAR on LTP. Bath application of ALCAR (100 μM) did not affect the degree of LTP in 6 month-old SAMR1. On the contrary, acute application of ALCAR improved the impaired LTP of SAMP8 in a dose-dependent manner. We also investigated the effect of ALCAR with aged SAMR1, but acute application of ALCAR did not alter the degree of LTP in 2 year-old SAMR1. Furthermore, to test if ALCAR restores the elevated oxidative stress level in SAMP8, we measured the level of reactive oxygen species using reactive oxygen metabolites assay, and reduction capacity using biological antioxidant potential assay. However, these indicators revealed that ALCAR could not restore the oxidative stress level elevating in SAMP8 compared with SAMR1. These results indicate that chronic supplementation of ALCAR can improve the age-dependent impairment of long-term synaptic plasticity with the exception of the case of aged individuals, but this improvement does not require the amelioration of oxidative stress level. Although we need to study the pharmacokinetics of ALCAR in more detail, our data about ALCAR in this study is expected for prevention of age-related dysfunction of the brain including dementia.

Disclosures: **S. Taniguchi:** None. **H. Takimoto:** None. **M. Hanafusa:** None. **H. Tsubone:** None. **D. Yamanaka:** None. **M. Kuwahara:** None. **K. Ito:** None.

Poster

686. Mechanisms of Synaptic and Neuronal Plasticity

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Topic: B.08. Synaptic Plasticity

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Title: Positive modulation of GABAA receptors by allopregnanolone moderates injury-induced, but not experience-induced, potentiation in the adult visual cortex in mice

Authors: *E. G. SERGEEVA[1], C. ESPINOSA-GARCIA[1], M. T. PARDUE[2,3], D. G. STEIN[1]; [1]Emergency Med., Emory Univ., Atlanta, GA; [2]Wallace H. Coulter Dept. of Biomed. Engin., Georgia Inst. of Technol., Atlanta, GA; [3]Atlanta VA Ctr. for Visual and Neurocognitive Rehabil., Atlanta, GA

Abstract: We used selective visual stimulation to examine enhancement in visual cortical response in adult mice with unilateral optic nerve crush (ONC). To investigate the role of inhibition in the experience-dependent potentiation of visual evoked response (VEP), we administered allopregnanolone (ALLO), a positive modulator of GABAA receptors. The left optic nerves were crushed in the ONC groups, and left intact in the Sham groups. ALLO (10 mg/kg) or vehicle was injected 1 hour after ONC and then on post-injury days (dpi) 3, 8, 13, and 18. VEP to pattern stimuli were recorded before ONC (Baseline, BL) and on dpi 2, 7, 12, 17, 22 and 30. Visual spatial frequency was assessed on BL and then on dpi 2, 6, 11, 16, 21 and 29 in an optokinetic tracking system. To evaluate levels of VGlut2 and GABAAR delta subunit on dpi 30, the animals were euthanized and their brains processed for immunohistochemistry. Sham animals showed a gradual increase in responses to stimulation of the contralateral eye throughout the experiment (146±6.4% of BL; $p<0.05$). The amplitude of VEP ipsilateral to the ONC eye, induced from the contralateral, intact eye, in the ONC/Veh group was higher than in Sham animals (197±12.7% of BL; $p<0.05$). VEP amplitude increase was associated with up-regulation of VGlut2 (increased excitatory synaptic activity) and down-regulation of GABAAR delta (decreased tonic inhibition) in the left primary visual cortex, which contains projections from the intact eye. ALLO produced lasting inhibitory effects in ONC animals as shown by attenuation of VEP amplitudes on dpi 17, 22 and 30 ($p<0.0001$) as well as by down-regulated levels of VGlut2 and up-regulated levels of GABAAR delta ($p<0.05$). In Sham animals ALLO did not affect either VEP amplitudes or VGlut2 and GABAAR delta levels, demonstrating that alterations in

GABAAR-mediated inhibition exert greater effects in injury-induced than experience-dependent VEP potentiation alone. Unilateral ONC increased spatial frequency thresholds in the intact eye, and ALLO induced an even larger enhancement ($p<0.0001$). A regression analysis revealed a U-shaped relationship between VEP amplitude and visual performance (R2 change for x2=0.100, $p=0.001$). These findings indicate that an optimal level of VEP potentiation is associated with higher visual performance in animals with unilateral ONC. Together, our data suggest potential clinical applications of ALLO for modulation of injury-induced, excessive and potentially maladaptive plasticity.

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Poster

686. Mechanisms of Synaptic and Neuronal Plasticity

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 686.05/H2

Topic: B.08. Synaptic Plasticity

Title: Neuronal activity regulates astrocyte proliferation

Authors: ***Y. CHEN**[1], C. FU[1], M. WEBER[2], H. LIN[2], H. NGU[2], J. KAMINKER[2], M. SHENG[2]; [1]IRCBC, SIOC, Chinese Acad. of Sci., Shanghai City, China; [2]Genentech Inc, South San Francisco, CA

Abstract: Astrocytes play critical roles in brain development and function, but the mechanisms that regulate astrocyte proliferation are poorly understood. We report that astrocyte proliferation is bi-directionally regulated by neuronal activity via NMDA receptor (NMDAR) signaling in neurons. Using whole genome mRNA profiling, we found that a set of cell cycle-related genes were altered by prolonged treatment of hippocampal cultures with NMDAR antagonist AP5. These cell cycle-related genes were expressed in astrocytes rather than neurons. NMDAR inhibition suppressed astrocyte proliferation *in vitro* and *in vivo*, whereas evoked neuronal activity promoted astrocyte proliferation in an NMDAR-dependent manner. Additional mechanistic studies identified Prostaglandin-endoperoxide Synthase 2 as being critical for activity-regulated astrocyte proliferation.

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Poster

686. Mechanisms of Synaptic and Neuronal Plasticity

Location: Halls B-H

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Topic: B.08. Synaptic Plasticity

Title: A critical role of the E3 ubiquitin ligase IDOL in regulating synaptic strength, cortical circuit plasticity, and learning and memory

Authors: *M. MAROSI[1], J. GAO[2], C. JINKUK[2], C. PORTERA-CAILLIAU[1], P. TONTONOZ[2];
[1]UCLA Depts. of Neurol. and Neurobio., [2]Howard Hughes Med. Inst., UCLA, Los Angeles, CA

Abstract: The lipoprotein receptors like Very Low Density Lipoprotein Receptor (VLDLR), and apolipoprotein E receptor 2 (ApoER2), that bind APOE have recently been recognized as pivotal components of the neuronal signaling machinery. In addition to being receptors for ApoE, VLDLR and ApoER2 also bind the signaling protein Reelin with high affinity. The Reelin signaling pathway is essential for not only controlling neuronal positioning during brain development, but also for maintaining synaptic plasticity and enhancing learning/memory in the adult brain. Inducible Degrader of the LDL-receptor (Idol) is an E3 ubiquitin ligase that regulates cellular cholesterol levels through binding and targeting LDLR for lysosomal degradation. Here we report that Idol is highly expressed in neurons throughout the central nervous system (e.g., hippocampus, neocortex and cerebellum) where it exerts a dominant role in determining endogenous neuronal VLDLR and ApoER2 expression level. Idol knockout mice have significantly higher neuronal VLDLR and ApoER2 levels. Interestingly, Idol knockout mice showed reduced hippocampal CA1 long-term potentiation (LTP) and enhanced long-term depression (LTD), with normal synaptic transmission (53% reduction in LTP and 16% enhancement in LTD). These phenomena are associated with impaired function of learning and memory, whereby mice lacking Idol have impaired spatial learning in the Morris water maze (longer escape latency), and reduced cued fear conditioning (lower % time of freezing). To investigate the role of Idol in experience-dependent plasticity in primary somatosensory cortex, we used longitudinal optical intrinsic signal (OIS) imaging to delineate single whisker response maps in barrel cortex. Two weeks after plucking all but one of the whiskers on the contralateral snout, we found an 89 % increase in the size of the spared whisker map in young adult wild type mice. In contrast, Idol KO mice did not show an expanded map for the spared whisker. These findings demonstrated how Idol can regulate neuronal lipoprotein receptor expression and plays an important role in synaptic function, cortical plasticity, learning and memory and behavior.

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Poster

686. Mechanisms of Synaptic and Neuronal Plasticity

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Title: Activity-dependent modulation of hippocampal synaptic plasticity via PirB and endocannabinoids

Authors: *M. DJURISIC, B. K. BROTT, C. J. SHATZ;
Dept. of Biol. and Bio-X, Stanford Univ., Stanford, CA

Abstract: The threshold for Hebbian synaptic plasticity is modulated by prior patterns of activity. Endocannabinoid (eCB) signaling has been postulated to play a role in this process, but it is not well understood how prior activity engages this retrograde signaling mechanism. Here, we investigate a role for the innate immune receptor Paired Immunoglobulin-like Receptor B (PirB) in regulating use-dependent changes in LTP and LTD at CA3 to CA1 hippocampal synapses. PirB is expressed in forebrain pyramidal neurons, including hippocampus. Conditional excision of PirB (cKO) in excitatory neurons of CA3 and CA1 at P90 results in loss of synaptically induced LTD over a wide range of LTD-induction frequencies. LTP is also increased over 2 fold in cKO vs. WT at 90 min post-induction. Concomitant with the shift from LTD towards greater LTP, neurotransmitter release is also increased in cKO mice, as signaled by a change in paired-pulse ratio. Endocannabinoids are known to regulate neurotransmitter release via cannabinoid receptor type 1 (CB1R) located presynaptically; this regulation is use-dependent and modulates synaptic plasticity. In WT, blockade of CB1R with AM251 abolishes synaptically induced LTD, and also results in an enhanced LTP, thus phenocopying synaptic plasticity changes present in PirB cKO. Moreover, blockade of CB1R in cKO does not further increase the magnitude of LTP, consistent with PirB acting in the CB1R pathway. In addition to CB1R, group I mGluRs and NMDARs are all thought to be engaged in eCB-dependent signaling at adult CA3 - CA1 synapses during synaptically induced LTD. We found that NMDAR-dependent LTD induced by brief application of NMDA was also abolished in PirB cKO, while CB1R- and group I mGluR-dependent chemical LTDs were intact. Together, results suggest that PirB in

hippocampal pyramidal neurons contributes to an NMDA-dependent mechanism of endocannabinoid signaling, enabling the synapse to function across a wide range of stimulus intensities. We suggest that PirB is a part of an on-demand signaling network that dynamically regulates the threshold for Hebbian synaptic plasticity by adjusting neurotransmitter release according to prior activity.

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Poster

686. Mechanisms of Synaptic and Neuronal Plasticity

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 686.08/H5

Topic: B.08. Synaptic Plasticity

Title: Human Neural Plasticity in a Dish

Authors: ***Y. DONG**[1], S.-C. ZHANG[2];
[1]Waisman Ctr., Univ. of Wisconsin, Madison, WI; [2]Univ. of Wisconsin-Madison, Madison, WI

Abstract: Long-term potentiation/long-term depression (LTP/LTD), inferred from analysis on the animal brain or slices, are considered the cellular processes underlying learning and memory formation. They have not so far been demonstrated in human brain or neurons and there are no model systems to assess long-term synaptic plasticity of human neurons. By expressing the light-gated cation channel, channelrhodopsin (ChR2) in human embryonic stem cell-derived cortical glutamate neurons and co-culturing them with GABA neurons, we examined synaptic plasticity in response to different regimens of stimulations. High frequency light stimulation of ChR2-expressing glutamatergic neurons increased the frequency of mEPSCs recorded in non ChR2-expressing GABA neurons, indicating a facilitating action at the presynaptic terminals. When paired with post-synaptic depolarization, it significantly increased the amplitude of light-evoked EPSC which persisted during the period, indicating LTP. In contrast, low frequency light stimulation without the pairing protocol induced LTD. These effects were blocked by NMDA receptor antagonists, suggesting that the LTP/LTD induced in human neurons are NMDA receptor dependent. Our results indicate a similar human neural plasticity. Our co-culture system may be a useful model for assessing human neural plasticity in a dish.

Disclosures: **Y. Dong:** None. **S. Zhang:** None.

Poster

686. Mechanisms of Synaptic and Neuronal Plasticity

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Topic: B.08. Synaptic Plasticity

Support: NASA Grant NNX13AB66G

Title: Long-term effects of galactic cosmic radiation on neurogenesis, synaptic plasticity, learning and memory

Authors: *O. MIRY, K. R. GOPAUL, L. R. VOSE, G. SUBAH, X.-L. ZHANG, P. K. STANTON;
Cell Biol. and Anat., New York Med. Col., Valhalla, NY

Abstract: Low doses of galactic cosmic radiation (GCR), containing high atomic number and high energy (HZE) particles, have been measured and are expected during long-duration deep space missions. While research has attempted to characterize the immediate and short-term effects of GCR insults to the central nervous system, little is known about chronic, long-term sequelae which may impact cognitive function over time. In particular, given the predisposition of the hippocampus to GCR, we hypothesized that there could be chronic alterations in hippocampal adult neurogenesis, synaptic plasticity, and hippocampus-dependent learning and memory in mice following exposure to simulated GCR.
Young adult (3 month old) male and female mice received whole-body particle radiation from a ^{28}Si (300 MeV/u, 70 keV/μm) or ^{56}Fe (600 MeV/u, 180 keV/μm) source totaling 10, 50, or 100 centiGray [cGy], using the particle beam line facilities at the National Space Radiation Laboratory of Brookhaven National Laboratory, and sham irradiated mice served as controls. Effects of GCR 6-7, 12, and 20 months post ^{28}Si and ^{56}Fe exposure on synaptic plasticity were assessed by measuring the magnitude of theta-burst stimulus-induced or chemical cAMP-induced long-term potentiation (LTP) of synaptic transmission at Schaffer collateral-CA1 synapses in hippocampal slices *in vitro*.
Six, twelve, and twenty months post ^{56}Fe exposure, the magnitudes of stimulus-evoked and chemical LTP were both markedly larger in amplitude ($P < 0.05$, ANOVA). Consistent with these data, separate cohorts of ^{56}Fe-exposed mice examined 6, 12 and 20 months post-exposure exhibited faster learning acquisition in both Barnes maze and active avoidance shock platform, both hippocampus-dependent spatial memory tasks ($P < 0.05$, repeated-measures ANOVA). In contrast, stimulus-evoked, but not cAMP-induced, LTP in hippocampal slices from both male and female mice 7 months post ^{28}Si exposure was significantly smaller than sham-irradiated controls ($P < 0.05$, ANOVA). These reductions were associated with slower memory acquisition in female, but not male mice, compared to sham-irradiated controls 7 months post ^{28}Si, while

learning acquisition was faster 20 months post ^{28}Si ($P < 0.05$, repeated-measures ANOVA). Our data reveal differences in long-term cognitive effects of GCR depending upon the species of ion exposure. As advancements in technology allow for more long-term, farther reaching space explorations such as the proposed Mars swing-by, it becomes increasingly important to understand the long-term consequences of GCR exposure to critical brain functions such as learning, memory, cognition, and mood.

Disclosures: **O. Miry:** None. **K.R. Gopaul:** None. **L.R. Vose:** None. **G. Subah:** None. **X. Zhang:** None. **P.K. Stanton:** None.

Poster

686. Mechanisms of Synaptic and Neuronal Plasticity

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 686.10/H7

Topic: B.08. Synaptic Plasticity

Support: IBS-R001-D1

DA 020087

Title: LARGE play a key role in hippocampus-dependent long-term memory formation by tuning synaptic targeting of AMPA receptors

Authors: ***M.-G. KANG**[1], B. SEO[2], T. CHO[2], D. Z. LEE[3], B. Y. LEE[2], S.-W. KIM[2], K. A. CUNNINGHAM[4], K. T. DINELEY[4], T. A. GREEN[4], J.-C. BÉÏQUE[5], H.-S. SHIN[2]; [1]Inst. For Basic Sci. (IBS), KAIST, Daejeon, Korea, Republic of; [2]Ctr. for Cognition and Sociality, Inst. for Basic Sci., Daejeon, Korea, Republic of; [3]Dept. of Neurosci. and Cell Biol., [4]Ctr. for Addiction Res., Univ. of Texas Med. Br., Galveston, TX; [5]Dept. of Cell. and Mol. Med., Univ. of Ottawa, Ottawa, ON, Canada

Abstract: Mutations in the human *Large* gene result in severe intellectual disability as well as muscular dystrophy. How *Large* mutations lead to intellectual disability, however, is not clear. To address this question, we knocked *Large* gene down in the hippocampi of mice followed with behavioral and electrophysiological analyses *in vivo*. A series of learning and memory tests demonstrated that hippocampus-dependent long-term memory formation is impaired in the LARGE knockdown mice. However, their hippocampus-dependent short-term memory was intact. In the LARGE knockdown mouse, hippocampal long-term potentiation (LTP) was occluded due to the saturation of synaptic α-amino-3-hydroxy-5-methyl-4-isoxazole propionate receptors (AMPA-Rs). Consistently, our miniature excitatory postsynaptic current (mEPSC)

analyses demonstrated that LARGE downregulates synaptic targeting of the AMPA-Rs. Together, LARGE play a key role in hippocampus-dependent long-term memory formation by tuning synaptic targeting of AMPA-Rs in the hippocampus. Our study provides novel insights into the pathophysiology of intellectual disabilities in patients with muscular dystrophy.

Disclosures: **M. Kang:** None. **B. Seo:** None. **T. Cho:** None. **D.Z. Lee:** None. **B.Y. Lee:** None. **S. Kim:** None. **K.A. Cunningham:** None. **K.T. Dineley:** None. **T.A. Green:** None. **J. Béïque:** None. **H. Shin:** None.

Poster

686. Mechanisms of Synaptic and Neuronal Plasticity

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Topic: B.08. Synaptic Plasticity

Support: FAPESP 2012/23918-0

Title: Hippocampal-prefrontal oscillations and synaptic plasticity in adult rats with early-life status epileptics

Authors: ***R. N. RUGGIERO**, D. B. MARQUES, L. S. BUENO-JUNIOR, C. LOPES-AGUIAR, J. B. DE ROSS, M. T. ROSSIGNOLI, L. KANDRATAVICIUS, J. P. LEITE; Univ. of São Paulo, Ribeirao Preto, Brazil

Abstract: Early-life seizures are frequent and associated with cognitive and psychiatric impairments later in life. We hypothesized that these behaviorally subtle dysfunctions could manifest as connectivity alterations between hippocampal CA1 and prefrontal cortex (PFC) since they are pathologically involved in the psychiatric comorbidities of adult limbic epilepsy. Thus, we induced a two-hour status epilepticus (SE) through lithium-pilocarpine in P12 rats. Once adults, they were tested for spatial working memory (radial arm maze), exploratory behavior (open field), and sensorimotor gating (prepulse inhibition of the acoustic startle, PPI). They were finally submitted to an acute electrophysiological session, with PFC field responses being recorded upon electrical pulses into CA1, both before (30 min) and after (240 min) CA1 high-frequency stimulation (HFS). Our results show that SE rats had worse radial arm maze performance, hyperlocomotion on the open field, PPI deficits, and an interestingly stronger long-term potentiation after HFS. Of note, no spontaneous seizures occurred throughout the experiment, and no changes in neuronal density were detected with NeuN immunohistochemistry. In a second experiment, adult rats with early-life SE were chronically implanted with microwire bundles into CA1 and PFC for the recording of local field potentials

and single-unit activity during a 24 h free-behavior session. Neurophysiological states were separated into active awake, resting awake, slow wave sleep and rapid eye movement sleep based on electromyogram and hippocampal theta power (4-8 Hz). Ongoing analyses compare SE and controls according to the sleep architecture, as well as hippocampal-prefrontal spectral density/coherence and cross-frequency coupling. We expect these analyses to detail the plasticity scenario outlined in the first experiment, which: (1) indicates long lasting CA1-PFC alterations independent of neuronal loss or recurrent seizures, and therefore (2) reveals unapparent neurophysiological dysfunctions in adult rats with early-life SE.

Disclosures: **R.N. Ruggiero:** None. **D.B. Marques:** None. **L.S. Bueno-Junior:** None. **C. Lopes-Aguiar:** None. **J.B. De Ross:** None. **M.T. Rossignoli:** None. **L. Kandratavicius:** None. **J.P. Leite:** None.

Poster

686. Mechanisms of Synaptic and Neuronal Plasticity

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UBACyT 2014-2017. 20020130200283BA

PIP 2011-2014. PIP 2011- 11220100100169

UBACyT 2011-2014. 20020100100870

Title: NF-kappa B activation during appetitive and aversive long-term memory consolidation in the inhibitory avoidance paradigm in mice

Authors: *A. SALLES[1], M. KRAWCZYK[2], M. BOCCIA[2], M. BLAKE[2], A. ROMANO[1], R. FREUDENTHAL[1];
[1]Lab. de Neurobiologia de la memoria, IFIBYNE, CONICET, Buenos Aires, Argentina; [2]Dto. Farmacología, FFyB, UBA, Buenos Aires, Argentina

Abstract: NFkB is a transcription factor whose activation has been shown to be necessary for long-term memory consolidation in several species. NFkB is activated in the nucleus of cells in a specific temporal window during consolidation. Previous results showed that the transcription factor is also present at the synapse and is activated during consolidation at different time points than in the nucleus . Our work focuses on the mouse inhibitory avoidance learning paradigm. In

this paradigm mice are placed on an illuminated platform with an entrance to a dark compartment; the experimental group receives an electric shock when entering the dark compartment. Delay on entering the compartment is evaluated 48hrs later. The behavioral control group consists of mice who experience the same context as the experimental group but do not receive an electric shock; 48hrs later this group of mice do not show a delay to enter the compartment. Nonetheless when analyzing the activation of NFkB we found that this group of mice has elevated activation when comparing to naïve. Furthermore, animals who receive a shock without being exposed to the platform show no delay in entering the dark compartment 48hrs post training and no activation of NFkB. We argue that the mice who don't receive the shock might perceive it as a relief (an appetitive learning situation), where they associate the context with a safe place and that this information may not be discriminated by the hippocampus. This appetitive memory can be impaired with an NFkB inhibitor (Sulfasalazine) injected directly into the hippocampus. Moreover we explore the role of the amygdala in this paradigm for both the appetitive and aversive situations. This work aims to discuss these results and further investigate how appetitive and aversive memories are consolidated.

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Poster

686. Mechanisms of Synaptic and Neuronal Plasticity

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Program#/Poster#: 686.13/H10

Topic: B.08. Synaptic Plasticity

Title: Vagus nerve stimulation directs both cortical and subcortical placticity

Authors: ***M. S. BORLAND**[1], N. A. MORENO[2], E. P. BUELL[1], J. M. BUELL[1], I. I. KHAN[1], A. S. KHAN[1], N. N. HOUSHMAND[2], C. A. KELLY[2], E. K. JENSEN[2], A. M. CARROLL[2], X. SHEN[1], M. P. KILGARD[1];
[1]Univ. of Texas At Dallas, Plano, TX; [2]Univ. of Texas at Dallas, Richardson, TX

Abstract: Repeated pairing of a movement or a sensory event with vagus nerve stimulation (VNS) can reorganize cortical maps. Repeatedly pairing a tone with a brief period of VNS increases the proportion of primary auditory cortex (A1) responding to the paired tone (Engineer et al., 2011). We predicted that in addition to A1, VNS would reorganize posterior auditory field (PAF), anterior auditory field (AAF), and inferior colliculus (IC). Microelectrode maps were constructed from ten rats that had received VNS stimulation paired with a tone 300 times per day for 20 days, and 10 experimentally naïve control rats. In rats that received VNS tone pairing, a

significantly greater number of cortical neurons were tuned to frequencies near the paired tone frequency in A1, PAF, and IC compared to control rats. VNS paired rats had significantly fewer neurons tuned to the same range in AAF. This is the first study to show that VNS directed plasticity differentially alters different auditory cortex fields.
N.D. Engineer, J.R. Riley, J.D. Seale, W.A. Vrana, J.A. Shetake, S.P. Sudanagunta, M.S. Borland, M.P. Kilgard. Reversing Pathological Neural Activity Using Targeted Plasticity. Nature, 2011.

Disclosures: **M.S. Borland:** None. **N.A. Moreno:** None. **E.P. Buell:** None. **J.M. Buell:** None. **I.I. Khan:** None. **A.S. Khan:** None. **N.N. Houshmand:** None. **C.A. Kelly:** None. **E.K. Jensen:** None. **A.M. Carroll:** None. **X. Shen:** None. **M.P. Kilgard:** None.

Poster

686. Mechanisms of Synaptic and Neuronal Plasticity

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Topic: B.06. Neurotransmitter Release

Support: DoD Grant W81XWH-14-1-0301

Dystonia Medical Research Foundation

Title: Dystonia-causing mutation in the Tor1a gene does not change the number or ratio of presynaptically silent synapses of central neurons

Authors: ***N. C. HARATA**, H. KAWANO, S. IWABUCHI, Y. KAKAZU;
Dept Mol Physiol & Biophys, Univ. of Iowa, Iowa City, IA

Abstract: Dystonia is a hyperkinetic movement disorder and is characterized by sustained or intermittent muscle contractions that cause abnormal movements or postures. Patients with dystonia are subject to atypical neuronal functions in the brain, in the absence of neurodegeneration. Thus, it is generally thought that one of the main pathophysiological causes is abnormal synaptic transmission. However, it remains unclear whether dystonia is associated with an imbalance between excitatory glutamatergic and inhibitory GABAergic neurotransmitter systems. Here we report on a study testing whether the numbers of these synapses are affected in the context of dystonia. Specifically, we prepared primary cultures of hippocampal neurons from a knock-in mouse model of DYT1 dystonia, the most common form of inherited dystonia. The data obtained from heterozygous neurons were compared with those from wild-type counterparts. We evaluated the numbers of morphologically identified synapses on dendrites by

immunocytochemistry, using the glutamatergic presynaptic marker vesicular glutamate transporter 1 (VGLUT1), the GABAergic presynaptic marker vesicular GABA transporter (VGAT), and the dendritic marker microtubule-associated protein 2 (MAP2). We also evaluated the synapses for functionality by labeling nerve terminals with a marker of recycling synaptic vesicles (FM1-43). The non-recycling (non-functional) synapses are defined as presynaptically silent, and the fraction of them among the morphologically identified synapses (both functional and non-functional) has been shown to change in response to changes in neuronal network activity, in the hippocampus and cerebral cortex of wild-type animals. Comparison of the mutant neurons to wild-type controls revealed no difference in the numbers of morphologically identified glutamatergic or GABAergic synapses, or in the ratios of glutamatergic vs. GABAergic synapses in each imaged field. Moreover, the fractions of presynaptically silent synapses did not differ, for either glutamatergic or GABAergic systems. Our results demonstrate a lack of change in the fundamental parameters that define the balance between synaptic excitation and inhibition. They further indicate that potential changes in neuronal network activity might not be strong enough to induce plastic changes in presynaptically silent synapses, at least under our experimental conditions.

Disclosures: **N.C. Harata:** None. **H. Kawano:** None. **S. Iwabuchi:** None. **Y. Kakazu:** None.

Poster

686. Mechanisms of Synaptic and Neuronal Plasticity

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Topic: B.08. Synaptic Plasticity

Support: 1RO EY014074-19

Title: Abnormal Retina -Specific Segregation at the dLGN of the Flailer - a myosin 5a mutant mice.

Authors: *S. PANDIAN;
McGovern Inst. for Brain Res., MIT, Cambridge, MA

Abstract: MyosinVa (MyoVA), is a widely distributed vesicular-cargo-binding actin motor known to deliver the major scaffold complex for glutamate receptors to spine synapses. In Flailer (Flr) mutant mice the cargo-binding domain of myo5a is driven by the brain specific promoter of gnb5 (Jones et al 2000). When this truncated myo5a is expressed in 1:1 ratio reproducible abnormal behaviors are seen (eg; early seizure, anxiety, repetitive whole body grooming (Pandian et al in prep). In visual cortex neurons these mice have abnormally high AMPAR

miniature current frequencies and their eAMPA/eNMDA ratio is significantly larger than in WT strain. Flr shows no LTD at the layer 4 to layer 2/3 synapses although LTP is normal (Yoshii et al 2013). In developing rodent dorsal lateral geniculate nucleus (dLGN) the ipsilateral input arrives later than the contralateral input (Godement et al., 1984) and there is a competition between the left and right eye axons (Huberman et al., 2008). We hypothesized that if the retinogeniculate pathway also lacked LTD the ipsilateral territory might be unable to terminate in its normal region because the earlier innervating projections could not undergo LTD. Here we report that when both eyes of Flr pups are differentially labeled with CTB (555, 647) they show abnormally small and displaced ipsilateral zone while the contralateral projection occupies a larger territory. Monocular enucleation before eye-specific segregation in Flr causes the contralateral projection to spread throughout the dLGN with lowest density of input in region normally occupied by the ipsilateral eye. The ipsilateral projection is abnormally large with a shift towards the medial side of the nucleus. The results are consistant with our results that NMDAR LTD is also defective in the LGN of Flr and prevents displacement of early contralateral inputs by the later arriving axons.

Disclosures: S. Pandian: None.

Poster

687. Modulation of Neuronal Firing Properties II

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 687.01/H13

Topic: B.09. Intrinsic Membrane Properties

Title: Using CaMPARI to understand activity-dependent changes at the axon initial segment

Authors: *R. J. O'TOOLE, M. B. HOPPA;
Biol., Dartmouth Col., Hanover, NH

Abstract: The arrangement and combination of ion channels and proteins comprising the axon initial segment (AIS) define the capacity of a neuron to integrate synaptic input, set action potential threshold, and generate specific patterns and rates of firing. Research has shown that chronic depolarization or deprivation of synaptic input to neurons can lead to physical relocation or rearrangement of the AIS. This plasticity is thought to act as a homeostatic response to regulate overall neural excitability. Importantly, neurons in more physiological conditions experience less extreme changes to their synaptic input, and it is largely unknown if a similar plasticity occurs in response to functional changes in activity. In this study we examine how the AIS regulates excitability and firing patterns, and inspect how the AIS is modified by functional changes in action potential output.

Quantifying neurons across a spectrum of neural activity has posed a major technical challenge to neuroscientists. Here, we have developed a system utilizing the recently developed genetically-encoded calcium integrator, CaMPARI. This indicator undergoes a permanent shift from green to red fluorescent emission *only during the simultaneous presence of 405 nm light and neural activity*, and stands as a permanent, ratiometric marker of neural activity. Exposing primary neurons, transfected with CaMPARI, to flashes of 405 light in culture allows us to characterize the activity levels of individual neurons. Using this system, we explore how the molecular profile of the AIS is modified under physiological conditions, and whether individual neurons undergo activity-dependent changes to regulate their intrinsic excitability.

Disclosures: R.J. O'Toole: None. **M.B. Hoppa:** None.

Poster

687. Modulation of Neuronal Firing Properties II

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Topic: B.09. Intrinsic Membrane Properties

Support: NIH grant R01 DA035913

NSF GRFP Grant No. 1144247

Title: D3 dopamine receptors are expressed in a novel pyramidal prefrontal cell subclass

Authors: *R. L. CLARKSON, A. T. LIPTAK, K. J. BENDER;
Ctr. for Integrative Neuroscience, Dept. of Neurol., UCSF, San Francisco, CA

Abstract: Prefrontal circuits process input from the internal and external world and use these inputs to guide decision-making and subsequent goal-directed behavior. The ability of the medial prefrontal cortex (mPFC) to flexibly guide appropriate behaviors, including updating expectations of reward and punishment, is highly sensitive to neuromodulators such as dopamine. Therefore, cellular expression of dopamine receptors on prefrontal projection neurons will affect how dopamine can act on the prefrontal network and therefore regulate downstream behaviors.

Dopamine acts in part to regulate the activity of mPFC layer 5 (L5) principal cells via D1-family (D1/D5) or D2-family (D2/D3/D4) receptors. Recent work indicates that D1- and D2-receptors (D1R/D2R) are expressed in largely separate subclasses of L5 pyramidal cell and that these subclasses have unique electrophysiological features and projection patterns, and are modulated by dopamine via distinct mechanisms (Gee et al., 2012; Seong and Carter, 2012). How other

dopamine receptor classes are distributed in mPFC is unknown.
Here, we demonstrate that D3R-expressing (D3+) pyramidal neurons are electrophysiologically distinct from D1+ and D2+ neurons, and therefore likely represent a separate cell subclass. Further experiments reveal that though both D2R and D3R are of the same family, and are both Gi-coupled, they can have distinct roles in mPFC. First, D3Rs, but not D2Rs, act to regulate neuronal excitability by modulating calcium channels at the axon initial segment. Second, D3+ pyramidal neurons project intracortically, in contrast to the subcortically-projecting D2+ neurons (Gee et al., 2012). Thus, antipsychotic compounds with strong affinity for D2-family receptors may regulate intracortical networks via actions at the D3R.

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Poster

687. Modulation of Neuronal Firing Properties II

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Topic: B.09. Intrinsic Membrane Properties

Support: NIH Grant R37 NS17813

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IAP VII/19 - DYSCO

Title: Reliable neuromodulation in variable model neurons

Authors: ***G. DRION**[1], T. O'LEARY[2], E. MARDER[3];
[1]Electrical Engin. and Computer Sci., Univ. of Liege, Liege, Belgium; [2]Univ. of Cambridge, Cambridge, United Kingdom; [3]Brandeis Univ., Waltham, MA

Abstract: Neurons and circuits are subject to significant changes in their firing activity and responsiveness to external inputs via neuromodulation (Marder, 2012). Many nervous system functions depend on reliable neuromodulation, yet the physiological properties of neurons that are targeted by neuromodulators are often highly variable (Grashow et al., 2009 ; Marder et al., 2014). In this work, we use conductance-based neuron models to investigate the mechanisms by which reliable neuromodulation can occur in populations of neurons with highly variable conductance densities. We describe how variability in neuron intrinsic properties can be classified into two types: variability in neuron passive properties vs active properties. Neuromodulation or any pharmacological manipulation that would directly affect ion channels in neurons exhibiting both these variability types is likely to be unreliable. However, we show that

a simple mechanism involving feedback interactions between the modulator receptor, a second-messenger signal and ion channel densities ensures reliability of neuromodulation even in very heterogenous populations. The ingredients for these mechanism are found in most metabotropic receptor signaling cascades.

References Grashow R, Brookings T, Marder E (2009) Reliable neuromodulation from circuits with variable underlying structure. Proc Natl Acad Sci U S A. 106:11742-6.
Marder E (2012) Neuromodulation of neuronal circuits: back to the future. Neuron. 76:1-11.
Marder E, O'Leary T, Shruti S (2014) Neuromodulation of circuits with variable parameters: single neurons and small circuits reveal principles of state-dependent and robust neuromodulation. Annu Rev Neurosci. 37:329-46.

Disclosures: G. Drion: None. **T. O'Leary:** None. **E. Marder:** None.

Poster

687. Modulation of Neuronal Firing Properties II

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Title: Synaptic and intrinsic mechanism of CB_{2R} mediated reduction of VTA DA neuronal excitability

Authors: *M. GAO[1], B. LARSEN[1,2], F. GAO[1], D. CHEN[1], Z. HUANG[1], H. ZHANG[3], Y. HE[3], Z. XI[3], J. WU[1];
[1]Dept. of Neurobio., Barrow Neurolog. Institute, St. Joseph's Hosp. and Med. Ctr., Phoenix, AZ; [2]Basic Med. Sci., Univ. of Arizona Col. of Med., Phoenix, AZ; [3]Mol. Targets and Medications Discovery Br., Natl. Inst. on Drug Abuse, Baltimore, MD

Abstract: We have recently reported that activation of cannabinoid type 2 receptors (CB_2Rs) reduced dopamine (DA) neuron excitability in the mouse ventral tegmental area (VTA). Here, we elucidated the underlying mechanisms. Using cell-attached recording in VTA slices, bath-application of a CB_2R agonist (JWH133) inhibited VTA DA neuron firing in a concentration-

dependent manner. In addition to JWH133, five other CB_2R agonists exhibited similar inhibition. Under the patch-clamp whole-cell recording model, JWH133 (10 μM) inhibited miniature excitatory postsynaptic currents (mEPSCs) but not miniature inhibitory postsynaptic currents (mIPSCs). However, JWH133 did not inhibit evoked EPSCs or IPSCs. In freshly dissociated VTA DA neurons, JWH133 reduced the action potential (AP) firing rate, delayed AP initiation and enhanced AP after-hyperpolarization. In voltage-clamp recordings, M-type K+ currents were enhanced by JWH133. This effect was absent in $CB_2^{-/-}$ mice and abolished by co-administration of a selective CB_2R antagonist (AM630). In addition, CB_2R-mediated inhibition in VTA DA neuron firing can be mimicked by a M-curren t opener and blocked by a M-current blocker. Finally, CB_2R-cAMP signaling plays a role in modulating DA neuron excitability since enhancement of cAMP by forskolin reduced M-current and increased DA neuron firing. These results suggest that CB_2Rs modulate VTA DA neuron excitability through both synaptic and intrinsic mechanisms, including a reduction in mEPSCs and enhancement of M-currents.

Disclosures: **M. Gao:** None. **B. Larsen:** None. **F. Gao:** None. **D. Chen:** None. **Z. Huang:** None. **H. Zhang:** None. **Y. He:** None. **Z. Xi:** None. **J. Wu:** None.

Poster

687. Modulation of Neuronal Firing Properties II

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Topic: B.09. Intrinsic Membrane Properties

Support: NS044163 (NINDS)

VA Merit Review 5 I01 BX000386

Title: Effects of internal recording anion on neuronal properties and firing of a genetically identified layer 5 pyramidal cell type.

Authors: ***G. S. NEWKIRK**[1], D. PATHAK[2], N. C. DEMBROW[1,3], R. C. FOEHRING[2], W. J. SPAIN[3,1];
[1]Physiol. & Biophysics, Univ. of Washington, Seattle, WA; [2]Anat. and Neurobio., Univ. of Tennessee, Memphis, TN; [3]VA Epilepsy Ctr. of Excellence, Seattle, WA

Abstract: Intrinsic properties of neurons determine the integration of inputs from the dendrites to the soma and ultimately the output pattern of the neuron, thus understanding these properties is integral to understanding the function of neuronal circuits. Unfortunately, the most reliable methods of single-cell recordings used to measure these properties requires mimicry of the

internal composition of the cell, where physiological conditions are near impossible to reproduce. Previous work in hippocampal CA1 pyramidal cells (Kaczorowski et al. 2007: J Physiol 578: 799) examined the effects of recording stability and slow afterhyperpolarization (sAHP) plasticity with internal solutions containing either Kmethylsulfate (Kmeth) or Kgluconate (Kgluc). We used solutions based on these two anions to investigate differences in action potential firing in a subset of layer 5 pyramidal neurons in brain slices from the somatosensory and motor cortex of 4-8 wk old mice expressing *Etv*1-EGFP. Experiments were also conducted in the perforated-patch (Perf) configuration using gramicidin to gain access to the internal compartment while maintaining physiological internal solution integrity. We compared resting potential, input resistance, impedance from chirp responses, action potential and afterpolarization parameters (fAHP, mAHP, sAHP & fADP), as well as repetitive firing responses (including spike frequency adaptation) to DC current steps as well as fluctuating current steps with all three internals. Subthreshold properties and firing responses recorded with the Kgluc or Perf internal did not differ significantly whereas responses recorded with KMeth showed significant differences in afterpolarization wave forms and dramatic differences in firing (decreased steady firing rates and, and marked increase in spike frequency adaptation as well as depolarization block). During prolonged recordings (>30 min) in KMeth, the firing responses became more similar to what was observed when recording with KGluc or Perf internals. Interestingly, firing responses recorded in a different type of layer 5 pyramidal neuron that lacks a sAHP (Thy1-yfp-h) were similar with each of the recording conditions.

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Poster

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TR-SFB134 to PK

CONNECT to PK

Title: Serotonin modulates the intrinsic electrophysiological properties of locus coeruleus neurons

Authors: *S. BREMSER[1,2], L. PAEGER[1,2], P. KLOPPENBURG[1,2];
[1]Biocenter, Inst. For Zoology, Cologne, Germany; [2]Cologne Excellence Cluster on Cell. Stress Responses in Aging-Associated Dis. (CECAD), Univ. of Cologne, Cologne, Germany

Abstract: The Locus Coeruleus (LC) contains about 50% of all noradrenergic neurons of the brain. Since these neurons have extensive projections throughout the CNS, the LC has been associated with the control of many different behavioral and cognitive functions such as arousal, stress, attention and memory processes (J. Psychopharmacol., 2013, 27(8):659-93). As an important prerequisite to unravel the physiological role of the LC, it is crucial to understand the intrinsic electrophysiological properties of the LC neurons. In previous work, certain electrophysiological properties of these neurons have been characterized. However, since the experiments have been performed in various organisms using different recording techniques the current data might not be quite comparable. Here we used perforated patch-clamp recordings, which do not disturb intracellular signaling, to comprehensively characterize LC neurons intrinsic electrophysiological properties in mice. Overall we found a homogeneous population of neurons with similar intrinsic electrophysiological properties including input resistance, excitability and action potential waveform. All LC neurons generated very regular pacemaker-like activity. Synaptic isolation did not disrupt pacemaking but significantly increased the action potential frequency in all experiments. All recorded neurons showed delayed excitation after hyperpolarization and prolonged hyperpolarization after sustained excitation.
In rodents the LC receives serotonergic projections from the dorsal raphe nucleus and LC neurons are inhibited by serotonin application. The physiological relevance of this connection is still not fully understood, hence we tested the effect of serotonin on the electrophysiological properties of LC neurons. Here we found that LC neurons in mice showed a complex response to serotonin. On the one hand serotonin caused a concentration dependent hyperpolarization accompanied by a reduction in action potential frequency, and a decrease in cell input resistance. On the other hand, the excitability in presence of serotonin was drastically increased. Together this might improve the signal to noise ratio in the LC, sharpening the noradrenergic signaling deriving from LC neurons. Our results confirm that serotonin modulates LC neurons, but also suggest that the modulatory effects are more complex than previously thought.

Disclosures: **S. Bremser:** None. **L. Paeger:** None. **P. Kloppenburg:** None.

Poster

687. Modulation of Neuronal Firing Properties II

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Title: Loss of Navβ4-mediated regulation of the resurgent and transient sodium current disrupts repetitive firing in cerebellar Purkinje neurons and impairs balance and motor coordination

Authors: *J. L. RANSDELL[1], W.-L. LO[2], P. M. ALLEN[2], J. M. NERBONNE[1];
[1]Departments of Developmental Biol. and Med., [2]Dept. of Pathology and Immunol., Washington Univ., Saint Louis, MO

Abstract: The resurgent component of the voltage-gated sodium (Nav) current, I_{NaR}, provides a depolarizing drive during the repolarization phase of action potentials and is thought to be vital in sustaining high rates of firing in many types of mammalian neurons. Previous work has linked the generation of I_{NaR} to the expression of the sodium channel beta four (Navβ4) auxiliary subunit. We used an Navβ4 'knockout' (*Scn4b*$^{-/-}$) mouse, as well as shRNA mediated acute 'knockdown' of *Scn4b*, to determine the effects of altered *Scn4b* expression on sodium currents in Purkinje neurons, as well as on the repetitive firing properties of these cells. In current-clamp experiments, we found that adult (6-8 week old) *Scn4b*$^{-/-}$ Purkinje neurons have reduced ($p<.01$) spontaneous and evoked firing rates. Acute knockdown of *Scn4b* in adult Purkinje neurons *in vivo* caused similar defects in high frequency firing. To determine if the defects in Purkinje neuron excitability impact motor performance, we used the elevated balance beam test. Here, animals were first trained to cross a narrow beam into an enclosed box and, following the training, the time it took to cross the beam and the number of foot slips along the way were measured to assess motor coordination and balance. These experiments revealed that adult *Scn4b*$^{-/-}$ animals have significant ($P<.05$) motor defects when compared to age matched WT animals. We used dynamic clamp to artificially insert I_{NaR} in mature *Scn4b*$^{-/-}$ Purkinje neurons and found that adding I_{NaR} increased spontaneous and evoked firing rates. Because of space-clamp limitations, we could not measure the magnitude of I_{NaR} in adult Purkinje neurons directly. We, therefore, measured I_{NaR} from Purkinje neurons acutely dissociated from postnatal day 12-18 (P12-P18) *Scn4b*$^{-/-}$ and WT animals. We found that I_{NaR} is significantly ($p<.05$) lower in P12-P18 *Scn4b*$^{-/-}$ Purkinje neurons, compared with WT Purkinje neurons. These voltage-clamp experiments also revealed that the peak transient sodium current density is significantly ($p<.05$) larger in P12-P18 *Scn4b*$^{-/-}$ Purkinje neurons than in WT Purkinje neurons. Interestingly, and in contrast to the attenuation in firing rate found in adult Purkinje neurons lacking *Scn4b*, repetitive firing rates in P14-P15 *Scn4b*$^{-/-}$ and WT Purkinje neurons were indistinguishable. Experiments are under way to determine the developmental expression of sodium channel alpha and beta subunits which may contribute to the observed age-dependent differences in the functional impact of the loss of *Scn4b* in cerebellar Purkinje neurons.

Disclosures: **J.L. Ransdell:** None. **W. Lo:** None. **P.M. Allen:** None. **J.M. Nerbonne:** None.

Poster

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NMSS RG 4924A1/1

Title: Identifying the passive membrane properties of myelinated axons

Authors: C. C. COHEN[1,2], M. A. POPOVIC[1], J. KLOOSTER[1], *M. H. KOLE[1,2];
[1]Netherlands Inst. for Neurosci., Amsterdam, Netherlands; [2]Cell Biol., Univ. of Utrecht, Utrecht, Netherlands

Abstract: Myelination of axons by glial cells is fundamental to the rapid conduction of action potentials (APs), providing reliable and long-range signaling in the nervous system. The electrical characterization of the myelinated axon was established in the first half of the 20th century, wherein multiple layers of myelin membrane tightly wrap axons between nodes of Ranvier to create a high-resistance axo-myelin membrane combination. In this paradigm most internodal current flow is restricted to the axon core, allowing little current to leak along the internode. While some experimental studies suggest the internode is leakier than previously thought, specific membrane properties of the axo-myelin circuit for central nervous system axons remain to be identified. Here, we made multi-site whole-cell recordings of myelinated axons of large layer 5 pyramidal neurons in the somatosensory cortex and examined the properties of passive voltage attenuation between the soma and axon cut ends (up to ~800 µm distance). Together with the morphology of recorded cells we developed cable models in which the specific axon and myelin membrane parameters were constrained to the experimentally recorded voltage responses. Results show that the optimized axo-myelin transverse resistance is much lower than expected according to the classic studies (21 kΩ cm^2; n = 6 axon models). Furthermore, based on the myelin ultrastructure identified with electron microscopy of the same recorded cells, we found a low specific resistance per myelin membrane (on average 7.85 kΩ cm^2, range: 1.59-17.7 kΩ cm^2, n = 4). The optimized cable models of myelinated axons further predicted a slower rise time of fast AP-like potentials within internodes relative to adjacent nodes of Ranvier. To test this experimentally, we made high-temporal resolution voltage-calibrated optical recordings of the axolemma in nodal and internodal regions. The results confirmed that nodal AP waveforms are threefold faster than those at preceding or proceeding internodes (12 nodes of 7 axons, P < 0.003). Taken together, these preliminary data indicate that transverse axo-myelin resistance is lower than expected according to classic electrophysiological studies of the myelinated axon.

Disclosures: **C.C. Cohen:** None. **M.A. Popovic:** None. **J. Klooster:** None. **M.H. Kole:** None.

Poster

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Topic: B.09. Intrinsic Membrane Properties

Support: NIH Grant NS062771

NIH TG 1R25GM109439-01A1

Title: Intrinsic excitability of cerebellar Purkinje cells is modulated by cholinergic inputs

Authors: G. WATKINS, *C. HANSEL;
Neurobio., Univ. of Chicago Dept. of Neurobio., Chicago, IL

Abstract: Synaptic plasticity has long been considered to be the fundamental mechanism underlying learning. Recently, however, changes in the intrinsic excitability of neurons in response to behavioral and electrophysiological stimuli have been identified as an important complementary process. In cerebellar Purkinje cells, intrinsic plasticity has been shown to be dependent on the activity of calcium-regulated potassium (SK) channels; additionally, in the hippocampus, signaling through muscarinic acetylcholine receptors (mAChRs) has been confirmed to be a regulator of SK channels. In order to identify the role of intrinsic excitability in the cerebellum, and to determine whether cholinergic signaling might have a similar function in modulating this effect, cerebellar slices were prepared from adult (P21-42) mice, and whole-cell patch-clamp recordings were performed from Purkinje cells in the vermis of the vestibular cerebellum (lobules IX/X), which contains a high concentration of mAChRs. We show that evoked spike activity is significantly increased following an induction protocol involving either somatic depolarization (SD; spike count increased to 159.3 ± 11.5% relative to baseline; n=9; $p < 0.05$) or a burst of parallel fiber synaptic input (Syn; 137.4 ± 11.6%; n=11; $p < 0.05$). While bath application of mAChR agonist oxotremorine-m in the absence of an induction protocol was also sufficient to increase Purkinje cell firing rate relative to the control (oxo-m; 115.3 ± 6.9%; n=7; $p < 0.05$), the effect was not as strong as either induction protocol on it's own. However oxo-m application significantly enhanced the response to the synaptic protocol (Syn+oxo-m; 172.1 ± 14.8%; n=6; $p < 0.05$), suggesting that mAChRs may have a function in modulating Purkinje cell excitability following synaptic input in the cerebellum. Taken together, these data indicate that intrinsic plasticity may play a role in cerebellar learning, and that cholinergic signaling may contribute to this mechanism.

Disclosures: **G. Watkins:** None. **C. Hansel:** None.

Poster

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Topic: B.09. Intrinsic Membrane Properties

Support: NNSFC Grant 31571222

Title: Characterization of serotonergic neurons in the medulla of epet-eyfp mice

Authors: ***Y. DAI**[1], S. YANG[1], K. CHEN[1], R. GE[2], Y. CHENG[1], N. SONG[1], X. GE[1];
[1]East China Normal Univ., 500 Dongchuan Road, Shanghai, China; [2]Inst. of Physical Educ., East China Jiao Tong Univ., Nanchang, China

Abstract: It has been shown that stimulation of the ponto-medullary junction, where serotonergic neurons originate, produces locomotion in the isolated neonatal rat brainstem-spinal cord preparation. 5-HT neurons in this region play an essential role in generating locomotion. However, the intrinsic membrane properties of these neurons remain unclear. Using ePet-EYFP mice in which EYFP was expressed in 5-HT neurons we were able to perform whole cell patch-clamp recordings on EYFP+ neurons of brainstem slices (PND 1 - PND 15). 5-HT neurons in medulla could be classified into three types: single (type 1), phasic (type 2) and tonic (type 3) firing based on their response to depolarization of step currents. Of 119 serotonergic neurons recorded from the parapyramidal region (PPR), 13% of the neurons were type 1, 9% type 2 and 78% type 3, whereas in total of 67 serotonergic neurons of the midline raphe nuclei (MRN), 18% were type 1, 6% type 2 and 76% type 3. Membrane properties measuring from 5-HT neurons in PPR (n=119) and MRN (n=67) included resting membrane potential (PPR -63.3±6 mV; MRN -65.9±6 mV), input resistance (PPR 1150.3±618 MΩ; MRN 1312.4±712 MΩ), membrane time constant (PPR 41.5±19 ms; MRN 47.5±23 ms), rheobase (PPR 30.1±23 pA; MRN 31.7±25 pA), voltage threshold (PPR -32.9±7 mV; MRN -32.6±8 mV), action potential height (PPR 38.8±9 mV; MRN 39.9±10 mV) and afterhyperpolarization depth (PPR 19.8±8 mV; MRN 21.1±10 mV). Except for resting membrane potential no significant difference was shown in the properties between the PPR and MRN 5-HT neurons. Fluorescent dying (2% cascade blue) was added into the electrodes to label the cells for morphological analysis. 5-HT neurons could be divided into oval and stellate categories according to somatic shapes or bipolar and multipolar groups based on the number of stem dendrites. Despite different from morphology 5-HT neurons were not substantially different in either membrane properties or morphological parameters (somatic diameter and spherical area). Hyperpolarization-activated inward current and persistent

inward current were found in both PPR and MRN 5-HT neurons. And furthermore, the tetrodotoxin and dihydropyridine resistant persistent inward current (TDr-PIC) was also expressed in these neurons. Bath application of 10-20 μM 5-HT enhanced the TDr-PIC or lowed the voltage or current threshold for eliciting plateau potential induced by TDr-PIC. Some neurons exhibited membrane oscillation or bursting which could be facilitated by ACh (10-30 μM). NMDA (10-30 μM) depolarized 5-HT neurons while ACh caused varying effects on them. This study provides insight into intrinsic properties of 5-HT neurons in the medulla of ePet-EYFP mice.

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Poster

687. Modulation of Neuronal Firing Properties II

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Program#/Poster#: 687.11/H23

Topic: B.09. Intrinsic Membrane Properties

Title: Roles of TRPC3 channels in nigral dopamine neurons; pacemaking and activity-dependent regulation of basal firing rate

Authors: ***K. UM**[1], L. BIRNBAUMER[2], H. KIM[1], M. PARK[1];
[1]Sch. of Medicine. Sungkyunkwan Univ., Suwon-City, Korea, Republic of; [2]BIOMED, Sch. of Med. Sciences, Catholic Univ. of Argentina, Buenos Aires, Argentina

Abstract: Dopamine neurons in the substantia nigra pars compacta (SNc) are slow pacemakers that generate spontaneous action potentials (APs) regularly. Although spontaneous action potentials are essential for maintenance of background dopamine levels and proper functioning of basal ganglia, it is not clear which channels are responsible for pacemaking and determine basal firing rate in the SNc dopamine neurons. In this study, we report that TRPC3 channels drive pacemaking and regulate basal firing rate via type 1 metabotropic glutamate receptors (mGluR1) in the SNc dopamine neurons. Specific TRPC3 channel blockers, pyr10 and pyr3, stopped spontaneous firing and Ca2+ oscillations in dopamine neurons. However, spontaneous firing was conserved in dopamine neurons of TRPC3 knockout (KO) mice and there was no significant difference in spontaneous firing rates between the TRPC3 KO and wild type mice. Application of pyr10 did not affect spontaneous firing and Ca2+ oscillations in TRPC3 KO mice, suggesting that pyr10 blocked spontaneous firing by specifically blocking TRPC3 channels in normal dopamine neurons. TRPC3 channel blockade with pyr10 hyperpolarized membrane potentials, but somatic current injection regenerated pacemaker activity again, suggesting that

TRPC3 channels are constitutively active and help to maintain membrane potential depolarized within pacemaking ranges. In addition, stimulation of mGluR1 induced sustained Ca2+ influxes together with enhancement of spontaneous firing. When TRPC3 channels were blocked by pyr10, mGluR1-induced slow Ca2+ influxes were dramatically reduced. Furthermore, in TRPC3 KO mice, firing rates were not significantly enhanced by activation of mGluR1 in SNc dopamine neurons. From these experimental results, we conclude that TRPC3 channels are not only essential for pacemaking, but also determine basal firing rate in mGluR1-dependent ways in SNc dopamine neurons.

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Poster

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WGL Special Project LIN 2013

Title: Cav3.2 T-type calcium channels modulate burst firing in mature granule cells of the dentate gyrus

Authors: *J. LOPEZ-ROJAS, M. DUMÉNIEU, M. HEINE, M. R. KREUTZ, A. BIKBAEV; Leibniz Inst. for Neurobio., Magdeburg, Germany

Abstract: Mature granule cells of the dentate gyrus exhibit sparse firing of action potentials in physiological conditions. Interestingly, in the rare case of activation they show burst firing that is believed to be crucial for communication with their postsynaptic CA3 targets. The mechanisms underlying this bursting pattern in mature granule cells are not known. In the dentate gyrus T-type calcium channels contribute to a reduction of the threshold for action potential generation in immature but not in mature granule cells. We hypothesized that T-type calcium channels might have a different function in mature cells by mediating their characteristic bursting behaviour. We performed patch-clamp recordings from mature granule cells of the dentate gyrus of rats and mice and found that pharmacological blockade of T-type calcium channels indeed impaired burst

firing. We found similar results when we isolated the neurons from the network by blocking glutamatergic and GABAergic transmission showing that contribution of T-type channels to the burst firing represents an intrinsic mechanism. We also studied the contribution of axonal and dendritic calcium currents. T-type-mediated currents were observed in the axon initial segment of mature granule cells as well as in their medial-distal dendrites. From the different T-type channels subtypes, Cav3.2 shows a strong expression in the dentate gyrus and a physiological/pharmacological profile matching the observed features of mature cells bursting. We therefore assessed the consequences of the deletion of the Cav3.2 gene on bursting by using Cav3.2 constitutive knock-out mice. Interestingly, Cav3.2 knock-out mice fired mainly tonic spikes with a clear impairment in their ability to generate bursts of action potentials when compared to controls. These results show that Cav3.2 channels are strong modulators of bursting in mature granule cells of the dentate gyrus. Thus, Cav3.2 channels might be considered as a critical molecular switch that would allow mature granule cells to be effective in transmitting information to the CA3 pyramids. Modulation of these channels by experience or post-translational modifications might have important consequences to pattern separation/completion tasks as well as other dentate and CA3-mediated behaviours.

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Poster

687. Modulation of Neuronal Firing Properties II

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Topic: B.09. Intrinsic Membrane Properties

Support: JSPS Young Scientists (A) 26710002

Kowa Foundation

Hakubi Project (Kyoto Univ.)

Title: Microglial activation induces the excitability plasticity in CNS neurons

Authors: *G. OHTSUKI[1], M. KIM[2];
[1]Hakubi-center / Dept. of Biophysics, Grad. Sch. of Sci., [2]Dept. of Mol. and Cell. Physiol., Kyoto Univ., Kyoto, Japan

Abstract: Microglia is the resident immune cells in the brain, which show inflammatory responses against the immune challenge caused from the bacterial infections, stroke, hypoxia,

autoimmune or neurodegenerative diseases. Activated microglia are involved in the synaptic elimination, synaptic efficacy and synaptic plasticity induction. However, whether the activated microglia alter the non-synaptic neuronal excitability plasticity remains unknown.
Here, we show activation of microglia using the bacterial endotoxin (lipopolysaccharide, LPS) modulates the neuronal excitability in two brain regions. First, in the cerebellar Purkinje neurons, exposure of LPS increased the firing frequency long-lastingly, which prevented from inducing the excitability plasticity by additional 5Hz somatic depolarization. LPS-induced firing increase was suppressed by under the existence of Apamin, the antagonist of small conductance Ca2+-activated K+ channels (SK channels), suggesting the LPS-induced firing increase was mediated through the SK2 channel down-regulation as shown that involved in the firing increase plasticity in Purkinje cells (Belmeguenai et al., 2010, JNS; Ohtsuki et al., 2012, Neuron; Grasselli et al., 2016, Cell Rep.). In contrast, the LPS decreased in firing frequency in layer 5 pyramidal neurons of the medial prefrontal cortex (mPFC). This decrease of pyramidal neurons were prevented by the intracellular application of the phosphatase inhibitor. Further, LPS-induced firing frequency decrease was in large part mediated by the up-regulation of the SK channel functions. And, we also analyzed inflammatory cytokine levels during microglial activation. Thus, these results indicated that the LPS-induced microglial activation modulates the neuronal excitability in Purkinje neurons and pyramidal neurons in opposite directions through SK channel functional changes.

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Poster

687. Modulation of Neuronal Firing Properties II

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Support: Israel Science Foundation (grant No. 1302/14)

Title: Sodium fluxes and cortical axonal excitability in penumbra following acute ischemic stroke

Authors: *O. STOLER[1], M. BOYKO[2], V. ZVENIGORODSKY[2], I. SHELEF[1], I. A. FLEIDERVISH[1];
[1]Dept. of Physiol. and Cell Biol., [2]Soroka Med. Ctr., Ben Gurion Univ., Baar Shava, Israel

Abstract: In the center of the cortical ischemic territory, the countdown to neuronal death starts with the onset of “anoxic depolarization” wave minutes after the onset of ischemia. The resulting

ischemic core is surrounded by the wider penumbra region where neuronal function is impaired but that is potentially salvageable with timely therapeutic intervention. Recent evidence indicates that the penumbral neurons suffer from rapid, calpain-mediated proteolysis of the axon initial segment (AIS) cytoskeleton. This stroke-induced proteolysis must have important functional implications for distribution and kinetics of the AIS channels thus affecting the ability of a neuron to generate meaningful output. Here we explored the stroke-induced changes in Na^+ fluxes and excitable properties of Layer 5 pyramidal neurons using a combination of patch-in-slice recording and high-speed fluorescence imaging of Na^+-sensitive indicator SBFI. Cortical slices including injured and contralateral hemispheres were prepared from the brains of two month old rats, 3-4 hours after focal infarction was produced by permanent middle cerebral artery occlusion. Following the surgery, animals were awakened from anesthesia to confirm neurological deficits. The boundaries of the ischemic core and of penumbra were determined using the diffusion and perfusion-weighted MRI before the animals were sacrificed. Immunohistochemical analysis revealed that ankyrin G immunoreactivity was undetectable in the AIS of neurons within the penumbra, whereas control NeuN-stained neurons in contralateral hemisphere displayed robust ankyrin G staining. The peak amplitudes of the single AP elicited Na^+ transients in the AIS of the penumbral neurons were smaller, but their times-to-peak were significantly longer than in the contralateral hemisphere, indicating that the inactivation characteristics of the underlying channels were altered. Less than 10% of the Layer 5 pyramidal neurons in control hemisphere fired bursts of action potentials (APs) in response to somatic, brief depolarizing current pulses. In the penumbra, however, the fraction of bursters increased markedly, with more than half of the neurons responded to the current pulses with more than one AP. This firing behavior was associated with increase in the size of AP afterdepolarization which could be attributed to incomplete inactivation of partially proteolyzed Na^+ channels. The changes in the firing pattern of the surviving cells of the penumbra may play an important role in development of post-stroke neurological deficits as well as in generation of spreading depolarization waves and epileptiform discharges following ischemic injury.

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Poster

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Topic: B.09. Intrinsic Membrane Properties

Support: KAKENHI (26250003)

Title: Spontaneous emergence of highly active neurons in hippocampal primary cultures

Authors: ***M. OKADA**, C. KOBAYASHI, Y. IKEGAYA;
The Univ. of Tokyo, Tokyo, Japan

Abstract: Neurons communicate with others using spikes. In the neuron society, cells are highly heterogeneous in terms of their activity level; only a few neurons exhibit extremely high firing rates, whereas many other neurons are nearly inactive. Although highly active neurons are not dominant in numbers, their spikes contribute the majority of the total spike number in the brain, and thus, they may have a great impact on information processing, compared to those emitted by other "normal" neurons. However, little is known about how highly active neurons emerged and are maintained in neuronal networks. We optically detected highly active neurons, using Arc-dVenus transgenic mice, which express the fluorescent protein dVenus under the promoter of *arc*, an immediate early gene that is induced by neuronal activation. We prepared primary cultures of hippocampal neurons from Arc-dVenus mice and continuously observed their dVenus fluorescence for about 50 days. Under spontaneous default-mode conditions, dVenus-positive neurons started to appear on approximately 20 days in vitro and gradually increased during our observation periods. However, even on 49 days in vitro, only a few portion (less than 1%) of neurons were dVenus-positive. The number of dVenus-positive neurons increased after neuronal stimulation by a $GABA_A$ receptor antagonist. Patch-clamp recordings revealed that the dVenus-positive neurons show spontaneously higher firing rates than dVenus-negative neurons. Although a subset of dVenus-positive neurons drift over time, some neurons were dVenus-positive for a long time (up to 14 days). Moreover, there existed neurons that repeatedly expressed dVenus. These results suggest that highly active neurons are fixed over time to some extent. To investigate the spatial distribution of highly active neurons, we calculated geometric energy, a measure of spatial clustering. The geometric energy was significantly higher than randomly shuffled surrogates, suggesting that Arc-expressing neurons were not randomly distributed but rather tended to be clustered in specific loci. Our results suggest that highly active neurons constitute a functional subpopulation in the neuronal network. These fixed highly active neurons may primarily convey neural information.

Disclosures: **M. Okada:** None. **C. Kobayashi:** None. **Y. Ikegaya:** None.

Poster

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Alzheimer's foundation

Title: Calcium lowers neuronal excitability with a G-protein independent mechanism

Authors: *M. FORSBERG[1], A. BJÖREFELDT[2], H. SETH[2], E. HANSE[2];
[1]Neurosci. and Physiol., Univ. of Gothenburg, Gothenburg, Sweden; [2]Physiol., Neurosci. and Physiol., Gothenburg, Sweden

Abstract: The effect of extracellular calcium to decrease intrinsic excitability in neurons has been known since the 19th century. The dominating hypothesis to explain this phenomenon has ever since been the so-called "charge-screen effect", claiming that the positively charged calcium ion is attracted to the negatively charged cell membrane and there screens the membrane with its positive charge. Thereby the local electrical field within the membrane is shifted to a more hyperpolarized membrane potential. Thus extracellular calcium ions suppress action potential firing. Recently, other mechanisms involving the G-protein coupled calcium sensing receptor (CaSR) and a sodium leak channel (NALCN) has been proposed.
In this study we use patch-clamp recordings of rat hippocampal CA1 pyramidal neurons to explore how changes in extracellular calcium from 2 mM (the most commonly used concentration in experiments *in vitro*) to 1 mM (the concentration in cerebrospinal fluid) affect intrinsic properties. This reduction in calcium produced a hyperpolarization of the threshold for action potential firing (-47 ± 1.0 mV to -52 ± 1.1 mV, $p<0.0001$) but no depolarization but rather a slight hyperpolarization of the resting membrane potential (-55.7 ±1.9 mV to −57.2 ±2.2 mV, $p=0.016$). We also observed a decrease of the action potential amplitude (106 ± 1.0 mV to 99 ± 1.3 mV, $p<0.0001$) and a decrease of the maximum depolarization velocity (258 ± 20.3V/s to 307 ± 22.8 V/s, $p=0.0001$). The rheobase decreased to 67 ± 7% (n=10), when calcium was changed from 2 to 1 mM. Addition of the G-protein blocker GDPβS intracellularly (0.3 mM) did not significantly change this result (66 ± 9 %, n=6). We also tested a higher concentration of GDPβS (3.0 mM, n=7), but that did not significantly reduce the effect of reduced calcium on the rheobase (61 ± 4 %, $p = 0.48$).
Our results indicate that the effect of calcium on intrinsic excitability does not depend on G-proteins. The hyperpolarization of the threshold for the action potential without alteration of the resting membrane potential suggests a mechanism involving the gating properties of voltage-gated sodium currents rather than leak currents.

Disclosures: **M. Forsberg:** None. **A. Björefeldt:** None. **H. Seth:** None. **E. Hanse:** None.

Poster

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INSERM

Aix-Marseille University

Title: Activity-dependent plasticity of intrinsic excitability in CA1 pyramidal neurons *In vivo*

Authors: *P. J. MORGAN, J. EPSZTEIN;
Inst. de Neurobiologie de la Méditerranée, Marseille Cedex 09, France

Abstract: On exposure to a novel environment, a subpopulation of cells in CA1 will express place fields to create a spatial map of the environment. What determines which cells will be recruited to this population is not yet known, but there is evidence to suggest intrinsic excitability could have an important role. The identity of place versus silent cells was found to be predictable from their firing pattern in response to current injections prior to exploration of a novel environment (Epsztein et al 2011): highly bursting cells tended to become place cells, whereas regular firing cells were more likely to be silent. Furthermore, small, tonic depolarisations of the membrane can be sufficient to reveal spatially modulated firing in cells that were previously silent (Lee et al. 2012).

Plasticity of intrinsic excitability in CA1 neurons has been shown following various stimulation protocols in vitro (Daoudal et al., 2002; Wang et al., 2003; Fan et al 2005, Brager & Johnston 2007, Campanac et al 2008), and could potentially regulate the availability of cells to encode a new environment, but it has yet to be demonstrated in vivo.

In this work we have examined the effect of three different stimulation protocols on the intrinsic excitability of CA1 pyramidal cells in vivo. Using whole-cell recordings in anaesthetised rats and stimulating solely via the patch pipette, we applied either 1. A theta-burst protocol previously used in vitro (Fan et al. 2005), 2. A pulse train protocol shown to modulate intrinsic excitability in the somato-sensory cortex in vivo (Mahon & Charpier 2012), or 3. A theta frequency plateau-inducing protocol, as plateaus occur at theta frequency at the centre of place fields (Epsztein et al 2011, Bittner et al 2015).

To determine intrinsic excitability we analysed the current-firing rate relationship, the membrane input resistance, the action potential threshold and the burstiness of the firing pattern. We found that the theta-burst protocol decreased intrinsic excitability, reducing the firing rate output and burstiness. These effects were accompanied by a decrease in input resistance. Similarly, the pulse

train protocol also reduced the action potential output and burstiness, however without a detectable effect on input resistance. The plateau group showed no overall differences in firing rate or burstiness, but for individual cells there were large effects in either direction, and we are exploring the possibility of differential subpopulation responses.
Our results show that single cell stimulation can induce plasticity of intrinsic excitability in CA1 pyramidal cells in vivo. Further work will investigate the role this may play in spatial coding.

Disclosures: P.J. Morgan: None. **J. Epsztein:** None.

Poster

687. Modulation of Neuronal Firing Properties II

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 687.18/I4

Topic: B.09. Intrinsic Membrane Properties

Support: CONACYT-Mexico SLC Grant 239192

CONACYT-Mexico MAR Grant 261796

INFR-2012-01-187757

Title: Differential effects of abuse solvents on the intrinsic excitability of pyramidal neurons in the medial prefrontal cortex of adolescents rats

Authors: *M. ARMENTA-RESÉNDIZ[1], S. L. CRUZ[2], E. J. GALVÁN[2];
[1]CINVESTAV Sede Sur, México, Mexico; [2]CINVESTAV, IPN Ciudad de México, Mexico

Abstract: Industrial products misused for intentional inhalation contain different solvents among which aromatic hydrocarbons such as toluene, xylene and benzene, are the most commonly found. Chronic abuse exposure to toluene produces clear behavioral effects and severe cognitive deficits, but the physiological adaptations that occur in specific brain areas are largely unknown. On the other hand, cyclohexane is another solvent, also found in commercial products which does not share behavioral effects toluene, suggesting that it may act through different mechanism. This work examines the effects of intermittent chronic exposure to toluene and cyclohexane in intrinsic excitability of pyramidal neurons of the medial prefrontal cortex (mPFC) in adolescent rats. Individual groups of male Wistar rats were exposed for 30 min in a static exposure chamber to 8000 ppm of toluene or cyclohexane twice daily for 10 days, from postnatal day 22 to 35. Pyramidal neurons in layer V were recorded using the patch-clamp technique in whole-cell mode. Neither toluene not cyclohexane changed the resting membrane potential. Toluene but not cyclohexane, decreased neuronal input resistance and rehobase

current, as well as increased the rectification phase of the curve I/V. Both solvents increased neuronal firing frequency, but, this increase was only significant in animals exposed to toluene. On the other hand, none of the solvent altered statistically the kinetics of the action potential. The increased frequency produced by toluene was related to a reduction in the after hyperpolarization current ($_{I}$AHP). In conclusion, our results show that repeated toluene exposure increases the excitability of pyramidal neurons layer V in the mPFC of adolescent rats while cyclohexane had no significant effect.

Disclosures: **M. Armenta-Reséndiz:** None. **S.L. Cruz:** None. **E.J. Galván:** None.

Poster

687. Modulation of Neuronal Firing Properties II

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Program#/Poster#: 687.19/I5

Topic: B.09. Intrinsic Membrane Properties

Support: DGAPA-UNAM IA105816

Title: Modulation of neuronal excitability in neuromorphic devices

Authors: ***A. FRANCI**[1], F. CASTAÑOS[2];
[1]Dept. de Matemáticas, Univ. Nacional Autónoma De México, Coyoacán, Mexico; [2]Dept. de Control Automático, CINVESTAV, Cd.Mx., Mexico

Abstract: The transition between different neuronal excitability types plays a fundamental role in brain functions [Lee2012]. A prototypical example is the transition between a tonic mode and a rebound/bursting mode in the thalamus associated with modulation of arousal state.
The balance between the expression of different ion channels is a key player in modulating neuronal excitability, and the involved ion channels can exhibit highly different or almost redundant properties. We have shown that, despite the high dimensionality of the ion channel conductance parameter space, modulation of neuronal excitability depends on a small number of behavioral parameters defining *dynamic input conductances* [Drion2015a]. These objects are instrumental to analyze neuronal modulation and robustness in a principled way [Drion2015b]. Here we use them in a constructive way to design simple electronic devices that exhibit the same excitability, modulation and robustness properties of biological neurons. Variations in biological parameters can be mapped into variations in the circuit parameters to reproduce the same behavioral transitions observed in experiments.
We propose this circuit as a real world analog implementation of the biological transition between excitability types and discuss its potentiality from a fundamental as well as from an

applied perspective.
Bibliography
[Lee2012] S.-H. Lee and Y. Dan, Neuromodulation of Brain States, Neuron, 76(1): 209-222, 2012.
[Drion2015a] G. Drion, A. Franci, J. Dethier, and R. Sepulchre, Dynamic input conductances shape neuronal spiking, eNeuro, 2(2):e0031-14.2015, 2015.
[Drion2015b] G. Drion, T. O'Leary, E. Marder, Ion channel degeneracy enables robust and tunable neuronal firing rates, Proc Natl Acad Sci U S A, 112:E5361-70, 2015.

Disclosures: **A. Franci:** None. **F. Castaños:** None.

Poster

687. Modulation of Neuronal Firing Properties II

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Program#/Poster#: 687.20/I6

Topic: B.09. Intrinsic Membrane Properties

Support: JSPS KAKENHI 15K19989

JSPS KAKENHI 25670668

JSPS KAKENHI 26293343

Title: Hydrogen peroxide modulates neuronal excitability and membrane properties in ventral horn neurons of the rat spinal cord

Authors: M. OHASHI[1], *T. KOHNO[2], N. OHASHI[1], T. HIRANO[1], K. WATANABE[1], H. SHOJI[1], T. MIZOUCHI[1], N. ENDO[1];
[1]Niigata Univ. Sch. of Med. and Dent. Sci., Niigata City, Japan; [2]Niigata Univ. Grad Sch. Med. & Dent. Sci., Niigata, Japan

Abstract: Hydrogen peroxide (H_2O_2), a reactive oxygen species, is an important signalling molecule in synaptic and neuronal activity in the central nervous system; it is produced excessively in brain ischaemia and spinal cord injury. Although we have reported H_2O_2-mediated modulations of synaptic transmission in rat spinal ventral horn (VH) neurons, the effects of H_2O_2 on neuronal excitability and membrane properties remain poorly understood. Accordingly, these potential effects were investigated in the present study using a whole-cell patch-clamp approach in rats. We found that bath-application of H_2O_2 decreased neuronal excitability accompanied by decreased input resistance, firing frequency, and action potential amplitude and by increased rheobase. These H_2O_2-mediated changes were induced by activation of extrasynaptic, but not

synaptic, $GABA_A$ receptors. Indeed, GABAergic tonic currents were enhanced by H_2O_2. On the other hand, the amplitude of medium and slow afterhyperpolarisation (mAHP and sAHP), which plays important roles in controlling neuronal excitability and is mediated by small-conductance calcium-activated potassium (SK) channels, was significantly decreased by H_2O_2. When extrasynaptic $GABA_A$ receptors were completely blocked, these decreases of mAHP and sAHP persisted, and H_2O_2 increased excitability, suggesting that H_2O_2 per se might have the potential to increase neuronal excitability via decreased SK channel conductance. These findings indicate that activating extrasynaptic $GABA_A$ receptors or SK channels may attenuate acute neuronal damage caused by H_2O_2-induced hyperexcitability and therefore represent a novel therapeutic target for preventing and treating H_2O_2-induced motor neuron disorders.

Disclosures: **M. Ohashi:** None. **T. Kohno:** None. **N. Ohashi:** None. **T. Hirano:** None. **K. Watanabe:** None. **H. Shoji:** None. **T. Mizouchi:** None. **N. Endo:** None.

Poster

687. Modulation of Neuronal Firing Properties II

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Program#/Poster#: 687.21/I7

Topic: B.09. Intrinsic Membrane Properties

Support: ANR EbGluNet

ERC consolidator Neuropioneer

Title: Temporal origin specifies an electrophysiological subpopulation of glutamatergic cells in the adulte dentate gyrus

Authors: *L. SAVE, A. BAUDE, R. COSSART;
INSERM U901, Marseille Cedex 09, France

Abstract: Temporal and spatial embryonic origin critically determines cortical neuron diversity. The Dentate Gyrus is the entry gate to the hippocampal trisynaptic circuit and comprises two subtypes of principal glutamatergic neurons, the granule cells and the semilunar granule cells. Although the latter represent a very small minority of neurons characterized by a stereotyped dendritic morphology, they have recently been identified as essential components of information processing through the Dentate Gyrus circuit. Since both early born GABAergic and glutamatergic neurons in CA3 were shown to develop into neurons supporting major network functions, we asked whether an early temporal origin could similarly specify semilunar cell fate. Here we show that semilunar granule cells, classified by their morphological features, are

generated and mature earlier than the general granule cell population. Besides morphology, adult semilunar cells display characteristic electrophysiological features contributing to a lower excitability. Such electrophysiological traits differ from most neurons but are shared with early born granule cells. Therefore an early birthdate specifies adult neuronal physiology whereas additional factors may combine to further produce morphological identity.

Disclosures: L. Save: None. **A. Baude:** None. **R. Cossart:** None.

Poster

687. Modulation of Neuronal Firing Properties II

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Title: Human cerebrospinal fluid promotes neuronal viability and activity of hippocampal neuronal circuits *In vitro*

Authors: *G. CULLEY[1], M. PEREZ-ALCAZAR[1], T. LYCKENVIK[1], K. MOBARREZ[1], A. BJÖREFELDT[1], P. WASLING[1], H. SETH[1], F. ASZTELY[1], A. HARRER[2], B. IGLSEDER[2], L. AIGNER[2], E. HANSE[1], S. ILLES[1];
[1]Univ. of Gothenburg, Goeteborg, Sweden; [2]Paracelsus Med. Univ., Salzburg, Austria

Abstract: ***Background***
For decades it has been hypothesized that molecules within the cerebrospinal fluid (CSF) diffuse into the brain parenchyma and influence the function of neurons. However, the functional

consequences of CSF on neuronal circuits are largely unexplored and unknown. A major reason for this is the absence of appropriate neuronal *in vitro* model systems, and it is uncertain if neurons cultured in pure CSF survive and preserve electrophysiological functionality *in vitro.*

Methods and Results

We present an approach to address how human CSF (hCSF) influences neuronal circuits *in vitro*. We validate our approach by comparing the morphology, viability, and electrophysiological function of single neurons and at the network level in rat organotypic slice and primary neurons cultivated either in hCSF or in defined standard culture media.

Summary

Our data indicate that hCSF represents a physiological environment for neurons *in vitro* and a superior culture condition compared to the defined standard media. Moreover, this experimental approach paves the way to monitor the functional consequences of CSF-derived molecules on neuronal circuits.

Disclosures: G. Culley: None. **M. Perez-Alcazar:** None. **T. Lyckenvik:** None. **K. Mobarrez:** None. **A. Björefeldt:** None. **P. Wasling:** None. **H. Seth:** None. **F. Asztely:** None. **A. Harrer:** None. **B. Iglseder:** None. **L. Aigner:** None. **E. Hanse:** None. **S. Illes:** None.

Poster

687. Modulation of Neuronal Firing Properties II

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Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 687.23/I9

Topic: B.09. Intrinsic Membrane Properties

Support: NIH Grant R01 NS028901

Title: Control of action potentials by Ca^{2+}-induced Ca^{2+} release

Authors: *T. IRIE[1,2], L. O. TRUSSELL[3];
[1]Oregon Hearing Res. Ctr., Oregon Hlth. Sci. Univ., Portland, OR; [2]Div. of Pharmacol., Natl. Inst. of Hlth. Sci., Tokyo, Japan; [3]Vollum Inst. & Oregon Hearing Res. Ctr., Oregon Hlth. Sci. Univ., Portland, OR

Abstract: Intracellular Ca^{2+} stores in the endoplasmic reticulum (ER) play crucial roles in electrical activity and signal transduction in muscle and neurons. In many cases, the release of ER Ca^{2+} is controlled by ryanodine receptors, which are opened by Ca^{2+} from the cytosolic side and contribute to amplification of Ca^{2+} signaling; this is known as Ca^{2+}-induced Ca^{2+} release, or CICR. In the central nervous system, CICR plays roles in synaptic plasticity and neuronal excitability. However, other physiological roles in the brain are poorly understood. The dorsal

cochlear nucleus of mammals integrates auditory and non-auditory information, and is thought to contribute to monaural sound localization. This region harbors glycinergic cartwheel interneurons, which have long been known to possess abundant subsurface ER cisterns in their cell bodies, and prominent Ca^{2+}-dependent signaling as well.
We investigated CICR-induced changes in cartwheel cell firing in current clamp, and associated outward currents in voltage clamp, using patch-clamp recordings from brainstem slice preparations of P18-26 mice. We also performed intracellular Ca^{2+} imaging by using two-photon microscopy.
Blockade of CICR by ryanodine dramatically changed the spontaneous spike output of cartwheel cells, transforming predominantly spontaneous regular firing into burst firing, and lengthening the duration of spontaneous burst firing. In voltage clamp recordings in the presence of synaptic blockers, 10 to 20 mV depolarizations from -70 mV holding potential led to appearance of spontaneous miniature outward currents (SMOCs), which were inhibited by iberiotoxin (a BK Ca^{2+}-activated K channel blocker), indicating the SMOCs are mediated by the activation of BK channels. The SMOCs were also inhibited by omega-agatoxin 4A (a P/Q-type Ca^{2+} channel blocker) or by ryanodine, indicating that SMOCs are mediated by CICR events triggered by Ca^{2+} influx through P/Q-type Ca^{2+} channels that leads to BK activation. In order to determine the sites of CICR in cartwheel cells, we monitored intracellular Ca^{2+} transients. Action potential-evoked Ca^{2+} transients in immediately under somatic membrane were attenuated by ryanodine, whereas the Ca^{2+} transients in the axon initial segment or in dendrites were not affected, indicating action potential-evoked CICR is present only in cell bodies.
We propose that action potentials rapidly trigger BK channel currents through CICR, and that this process tunes the size and duration of burst firing in cartwheel cells.

Disclosures: T. Irie: None. **L.O. Trussell:** None.

Poster

687. Modulation of Neuronal Firing Properties II

Location: Halls B-H

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Program#/Poster#: 687.24/I10

Topic: B.09. Intrinsic Membrane Properties

Title: Propofol-mediated spike firing suppression is more sensitive in pyramidal neurons than in fast-spiking interneurons in the rat insular cortex

Authors: K. KANEKO[1], Y. KOYANAGI[1], *K. YAMAMOTO[3], Y. OI[1], M. KOBAYASHI[2]; [1]Anesthesiol., [2]Nihon Univ. Sch. of Dent., Tokyo, Japan; [3]Dept. of Pharmacology, Nihon Univ. Sch. of Dent., Tokyo, Japan

Abstract: Propofol is a major intravenous anesthetic, which facilitates $GABA_A$ receptor-mediated inhibitory synaptic currents. Moreover, propofol modulates several ionic currents including I_h, K^+, and voltage-gated Na^+ currents. These propofol-induced modulation of ionic currents results in hyperpolarization of the resting membrane potential, decreasing the input resistance, and suppression of repetitive spike firing in pyramidal neurons in the cerebral cortex. However, it has been unknown whether these ionic currents are modulated by propofol in fast-spiking and non-fast-spiking GABAergic interneurons, which differentially express these ionic channels. The present study aimed to examine whether pyramidal and GABAergic neurons are differentially modulated by propofol in the rat insular cortex. We performed multiple whole-cell patch-clamp recording from pyramidal and GABAergic interneurons that were classified into fast-spiking (FS) GABAergic interneurons and non-FS GABAergic neurons by their spike firing properties. We found that 100 μM propofol commonly hyperpolarized the resting membrane potential and decreased the input resistance in pyramidal, FS, and non-FS neurons. Hyperplarization-induced sag, which was prominently observed in pyramidal neurons but not in the most FS and non-FS neurons, was diminished by propofol. In addition, propofol (100 μM) potently suppressed, in most cases eliminated, the repetitive spike firing in pyramidal, FS, and non-FS neurons. Therefore, quantitative analyses of propofol-induced firing modulation were performed at low concentration of propofol (30-50 μM). We found that 30-50 μM propofol decreased the neural firing of pyramidal neurons but had little effect on that of FS and non-FS neurons, suggesting that pyramidal neurons were most sensitive to propofol among cortical neurons.

Disclosures: K. Kaneko: None. **Y. Koyanagi:** None. **K. Yamamoto:** None. **Y. Oi:** None. **M. Kobayashi:** None.

Poster

687. Modulation of Neuronal Firing Properties II

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Program#/Poster#: 687.25/I11

Topic: B.09. Intrinsic Membrane Properties

Support: Indiana University Start-up Funds

Title: Dissecting the functional impact of sphingosine-1-phosphate signaling on pain modulation in the central amygdala

Authors: B. E. MORK[1], R. R. DONAHUE[3], B. K. TAYLOR[3], *P. L. SHEETS[4,2];
[1]Dept. of Integrated Biomed. Sci., [2]Dept. of Biol. Sci., Univ. of Notre Dame, Notre Dame, IN;

[3]Dept. of Physiol., Univ. of Kentucky Med. Ctr., Lexington, KY; [4]Dept. of Pharmacol. and Toxicology, Indiana Univ. Sch. of Medicine-South Bend, South Bend, IN

Abstract: Inflammation leads to the production of the bioactive lipid sphingosine-1-phosphate (S1P) which sensitizes peripheral nociceptors via activation of one of five G-protein coupled receptors (S1P receptors 1-5). Despite the expression of these receptors in the CNS, the contribution of S1P signaling to supraspinal circuits relevant in pain processing remains poorly understood. The amygdala is a temporal lobe structure essential to the affective component of pain and stress-induced analgesia. S1P stimulates G-protein activity in the amygdala yet the functional effects of S1P signaling on projectionally defined neurons in the amygdala is lacking. The central nucleus of the amygdala (CeA) is the major output pathway of the amygdala and contains a major subpopulation of GABAergic neurons that modulate pain-related behaviors. Using anatomical labeling techniques in the mouse, we identified CeA neurons that send projections to the periaqueductal gray (PAG), a midbrain structure essential to endogenous analgesia. In acute brain slices, the effects of S1P on the intrinsic properties of retrogradely-labeled CeA-PAG neurons were measured using whole-cell electrophysiology. Preliminary results suggest that exogenous S1P hyperpolarizes CeA-PAG neurons leading to a decreased firing frequency. Surprisingly, addition of S1P receptor 1 (S1PR1) agonist SEW2871 depolarized CeA-PAG neurons thereby increasing firing frequency. This suggests that the decrease in excitability caused by exogenous S1P may be mediated by a different S1P receptor. We are currently injecting S1PR agonists and antagonists within the CeA to test the hypothesis that S1P signaling modulates hyperalgesia in an inflammatory pain model. Ultimately, findings from this work may uncover therapeutic targets within intersecting pain and emotional-affective circuity that could lead to novel approaches exploiting S1P pathways for treating both chronic pain and anxiety disorders.

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Poster

688. Epilepsy: Glia and Post-Seizure Mechanisms

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 688.01/I12

Topic: B.11. Epilepsy

Support: SIUC-SOM

RSG-SOM PRP

Title: Epileptic mice exhibit increased Kir6.2 in reactive astrocytes

Authors: *P. R. PATRYLO[1,2], A. A. SHARP[1,2], X. YAN[3], C. M. GRIFFITH[1];
[1]Dept of Physiol., [2]Anat., SIUC Sch. of Med., Carbondale, IL; [3]Dept of Anat. and Neurobio., Central South Univ. Xiangya Sch. of Med., Changsha, China

Abstract: Epilepsy is a devastating neurological condition characterized by recurrent spontaneous seizures. Approximately 30-40% of all epileptic cases are medically refractory to available therapeutics thus dictating the need to identify additional targets involved with epileptogenesis and ictogenesis. ATP sensitive potassium channels (K_{ATP}) may be such a target since 1) K_{ATP} channels contribute to the anti-convulsive effects of triamterene, the ketogenic diet and 2-deoxy-D-glucose (2-DG) and 2) defects in K_{ATP} channels (KCNJ11 mutations) underlie DEND syndrome which is characterized by developmental delay, epilepsy and neonatal diabetes. Subsequently, we hypothesized that alterations in K_{ATP} channels could occur in animal models of epilepsy, specifically the pilocarpine-mouse model of temporal lobe epilepsy. Adult male mice (C57Bl6/129; 2-4 months) were treated with methyl-scopolamine (1.2 mg/kg; IP) followed by either pilocarpine (295 mg/kg; IP) or vehicle and then behaviorally monitored to verify status epilepticus. Three to six months following the initial treatment, animals were behavioral monitored (8-10 hrs/week) to assess for spontaneous convulsive seizures. Mice were then sacrificed and their hippocampi were collected and fixed in 4% paraformaldehyde (epileptic pilocarpine-treated mice, n = 4; non-epileptic vehicle-treated controls, n = 4). Cresyl violet staining and immunohistochemistry (IHC) for BACE1 revealed that 100% of epileptic pilocarpine-treated mice, versus 0% of controls, exhibited extensive hippocampal cell loss and mossy fiber sprouting. To examine K_{ATP} channels, IHC for Kir6.2 (one of the pore-forming subunits of K_{ATP} channels) was performed. Saline treated controls exhibited Kir6.2 immunoreactivity (IR) in hippocampal pyramidal cells and dentate granule cells (primarily the cell body) which is similar to what has been previously reported (Zhou et al., 2002). Hippocampal interneurons were also Kir6.2-IR with very little astrocytic staining noted. Slices from epileptic pilocarpine treated mice exhibited a similar pattern of Kir6.2-IR in the remaining hippocampal neurons and strong Kir6.2-IR in the soma and primary processes of reactive astrocytes in both the grey and white matter. This aberrant increase of Kir6.2 in astrocytes was surprising since Kir6.2 has been suggested to be primarily neuronal. While the physiological consequences of this aberrant expression of Kir6.2 in astrocytes is unclear, it is interesting to note that modulation of K_{ATP} channels can affect astrocytic gap junctions which subsequently could affect hippocampal network activity.

Disclosures: P.R. Patrylo: None. **A.A. Sharp:** None. **X. Yan:** None. **C.M. Griffith:** None.

Poster

688. Epilepsy: Glia and Post-Seizure Mechanisms

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 688.02/J1

Topic: B.11. Epilepsy

Title: Blood brain barrier disruption: a potential mechanism for astrocyte dysfunction in epilepsy

Authors: *J. BONI, A. C. RANDOLPH, L. L. MCMAHON, M. L. OLSEN;
Univ. of Alabama At Birmingham, Birmingham, AL

Abstract: Kir4.1, a glial specific, inwardly rectifying potassium channel contributes significantly to astrocyte membrane properties and extracellular K+ homeostasis. Reduced protein expression and channel function is observed in nearly every CNS pathology and astrocyte specific rescue reduces neuronal hyperexcitability and dysfunction, leading to the notion that Kir4.1 may represent a novel therapeutic target. Recent work suggests Kir4.1 downregulation may be due, in part, to extravasation of serum components into the parenchyma upon blood-brain barrier (BBB) disruption. Using a pilocarpine model of status epilepticus and spontaneous recurrent seizures in adult male rats we observed a > 50% reduction in hippocampal Kir4.1 expression 24 hours post status epilepticus that was associated with hippocampal extravasation of Evans blue dye, used as an indicator of blood-brain barrier breakdown. Reduced Kir4.1 protein levels were maintained through the latent period, preceded the onset of spontaneous recurrent seizures and were maintained in epileptic animals. Treatment with Thiamet-G, a specific inhibitor of O-GlcNAcalyation (OGA) previously shown to be neuroprotective, one hour post status epilepticus rescued Kir4.1 levels to that of sham animals. Work underway is aimed at addressing 1) in the context of status epilepticus if BBB disruption is necessary for reduced Kir4.1 expression 2) if treatment with Thiamet-G rescue Kir4.1 protein levels at chronic time points after pilocarpine injection 3) if the rescue of Kir4.1 expression by Thiamet-G occurs by direct action on astrocytes or through attenuating BBB breakdown?

Disclosures: J. Boni: None. **A.C. Randolph:** None. **L.L. McMahon:** None. **M.L. Olsen:** None.

Poster

688. Epilepsy: Glia and Post-Seizure Mechanisms

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 688.03/J2

Topic: B.11. Epilepsy

Title: Role of COX-2 mediated endocannabinoid degradation in postictal hypoxia

Authors: *R. COLANGELI[1], J. S. FARRELL[2], K. ADDO-OSAFO[2], M. N. HILL[2], G. C. TESKEY[2];
[1]Dept. Cell Biol. and Anat., [2]Univ. of Calgary, Calgary, AB, Canada

Abstract: The post-ictal period is often associated with acute neurological dysfunction, such as sensory, cognitive, and motor impairments. By using several models of seizures and epilepsy our laboratory has characterized the occurrence of a long-lasting period of hypoperfusion and severe hypoxia (pO_2 below 10 mmHg) which begins immediately after seizure cessation and lasts over an hour in the hippocampus. Furthermore, this local phenomenon is associated with acute postictal behavioural dysfunction that is related to the brain structure involved in the electrographic seizure. Postictal hypoperfusion/hypoxia is completely prevented by the pre-administration, but not post-administration, of Cyclooxygenase-2 (COX-2) inhibitors, which suggests a chief role of COX-2 activation during the electrographic seizure. COX-2 is an inducible enzyme which catalyses the conversion of arachidonic acid to the vasoactive prostaglandins (PG). However, selective inhibition of downstream PGE2 and thromboxane synthesis did not prevent postictal hypoxia. Like COX-2-dependent PG synthesis, the two main endogenous cannabinoids anandamide (AEA) and 2- arachidonoylglycerol (2-AG) are synthesised and released during sustained neuronal activity and increased intracellular Ca^{++} concentration. Seizures cause an intense pattern of neuronal activity, which potently co-activate both the COX-2 and endocannabinoid systems. AEA and 2-AG are primarily inactivated by fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL), respectively. Importantly, it has been demonstrated that COX-2 represents an alternative pathway for the breakdown of endocannabinoids and generation of long-lasting vasoactive metabolites. Based on this evidence we hypothesised that the COX-2-derived endocannabinoid metabolites, PG-glycerol esters (PG-G) for 2-AG and PG-ethanolamides (PG-EA or prostamides) for AEA, might be responsible for the long-lasting severe postictal hypoxia induced by electrographic seizures. We expect that administration of a FAAH inhibitor (URB597) or MAGL inhibitor (MJN 110) may worsen postictal hypoxia by increasing COX-2-dependent endocannabinoids degradation, whereas administration of substrate-selective COX-2 inhibitors, which inhibit endocannabinoid and not arachidonic acid breakdown, (LM4131 and (R)-flurbiprofen) may prevent hypoxia following seizures. These experiments contribute to our understanding of the mechanisms of postictal

hypoperfusion/hypoxia and provide further preclinical evidence for the development of new endocannabinoid-based pharmacological tools for the treatment of epilepsy.

Disclosures: **R. Colangeli:** None. **J.S. Farrell:** None. **K. Addo-Osafo:** None. **M.N. Hill:** None. **G.C. Teskey:** None.

Poster

688. Epilepsy: Glia and Post-Seizure Mechanisms

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 688.04/J3

Topic: B.11. Epilepsy

Title: Status epilepticus triggers changes in microglia morphology that are associated with specific biochemical properties

Authors: S. K. WYATT, S. A. HERR, *A. L. BREWSTER;
Psychological Sci., Purdue Univ., West Lafayette, IN

Abstract: Long-lasting seizure activity (status epilepticus; SE) is often associated with hippocampal injury and the subsequent development of spontaneous seizures (epilepsy). One hallmark often associated with SE-induced hippocampal injury is activation and accumulation of microglial cells. Under pathological conditions (e.g. seizures) microglial cells develop an inflammatory phenotype that includes a change in morphology from highly ramified to hypertrophied and amoeboid, and the release of immunological messenger molecules such as cytokines. Recently, we reported SE-triggered changes in the morphology and accumulation of microglial cells in the hippocampus. These changes were evident between 1 to 14 days after SE, when microglia changed gradually from highly branched to amoeboid and accumulated in the CA1 hippocampal region. Interestingly, we found little correlation between accumulation of amoeboid microglia and the levels of multiple inflammatory cytokines using whole hippocampal homogenates. Thus, the objective of this study was to identify the cytokine profile expressed in the different morphological states of microglia after SE. SE was induced in rats using the chemoconvulsant pilocarpine and stopped after 1 hour with the anticonvulsant diazepam. Tissue was collected at 14-days after SE for immunohistochemistry. Antibodies against IBA1 or CD11b were used to identify microglia independently of their activation status. Microglial morphological properties were determined by using a 1-5 scale as follows: 1-ramified, 2-bushy/reactive, 3-hypertrophied, 4- amoeboid and 5-rod microglia. To understand the biochemical properties of these different morphologies of microglia, immunofluorescence was used with antibodies against inflammatory cytokines such as IL-1β, TNFα, IL-6, along with CD68 and MHCII. First, we found a significant increase in the total number of microglial cells in

the hippocampi of SE rats compared to the control group ($p<0.05$). Morphological analyses showed that in control hippocampi there was a higher number of ramified microglia compared to the other morphologies. In contrast, SE hippocampi displayed a significant increase in the numbers of amoeboid and rod-shaped microglia compared to the control ($p<0.05$) that correlated with a significant decline in ramified microglia. In addition, we found little to no cytokine expression in branched microglia and different cytokine profiles in hypertrophied, amoeboid, and rod-shaped microglia. These data suggest that SE triggers changes in microglia morphology as well as cytokine expression and that different microglia morphologies may have different functions.

Disclosures: **S.K. Wyatt:** None. **S.A. Herr:** None. **A.L. Brewster:** None.

Poster

688. Epilepsy: Glia and Post-Seizure Mechanisms

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 688.05/J4

Topic: B.11. Epilepsy

Support: Deutsche Forschungsgemeinschaft (STE 552/3-1)

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Bundesministerium für Bildung und Forschung (01EW1501B)

Title: Cytokine-induced uncoupling of hippocampal astrocytes as a cause of human temporal lobe epilepsy

Authors: *P. BEDNER[1], T. DESHPANDE[1], J. MÜLLER[1], D. KHAN[1], M. SYLVESTER[2], V. GIESELMANN[2], P. DE GRAAN[3], C. STEINHÄUSER[1];
[1]Inst. of Cell. Neurosciences, Bonn, Germany; [2]Inst. of Biochem. and Mol. Biol., Bonn, Germany; [3]Dept. of Translational Neurosci., Brain Ctr. Rudolf Magnus, Utrecht, Netherlands

Abstract: Glial cells are now recognized as active communication partners in the CNS, and this new perspective has rekindled the question of their role in pathology. Recently, we have compared functional properties of astrocytes in hippocampal specimens from patients with mesial temporal lobe epilepsy (MTLE) with and without hippocampal sclerosis (MTLE-HS). The data showed that the sclerotic human hippocampus is completely devoid of *bona fide* astrocytes and gap junction coupling, whereas coupled astrocytes were abundantly present in non-sclerotic specimens. Employing a new mouse model of MTLE-HS, we could also show that astrocytic uncoupling and the consequential impairment of K^+ clearance temporally precede

neuronal death and the onset of spontaneous seizure activity (Bedner et al. 2015, Brain 138:1208-22). Here we report that similar uncoupling occurs in another seizure model, i.e. hyperthermia-induced febrile convulsions, providing strong evidence that this dysfunction represents a fundamental mechanism in epileptogenesis. Our current results suggest that the inhibition of astrocyte coupling during the early phase of epileptogenesis is not caused by reduced levels of gap junction proteins but by changes in the phosphorylation status of Cx43, as evidenced by Western blot and mass spectrometric analyses. Proinflammatory molecules appeared to mediate this posttranslational modification of Cx43, since astrocyte uncoupling was absent in toll-like receptor 4 knockout mice, could be induced by cytokines *in situ* and *in vivo,* and could be rescued by intraperitoneal injection of Xpro1595, a specific inhibitor of soluble TNFα, or of the antiepileptic drug levetiracetam. These data challenge the commonly accepted neurocentric view of epileptogenesis and demonstrate that astrocytes may be the prime cause of this condition.

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Poster

688. Epilepsy: Glia and Post-Seizure Mechanisms

Location: Halls B-H

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Program#/Poster#: 688.06/J5

Topic: B.11. Epilepsy

Title: Prolonged seizures trigger activation of the classical complement pathway in the hippocampus

Authors: *N. D. SCHARTZ, A. L. BREWSTER;
Dept. of Psychological Sci., Purdue Univ., West Lafayette, IN

Abstract: Prolonged continuous seizure activity (status epilepticus; SE) can severely disrupt the hippocampal circuitry and promote the development of spontaneous recurrent seizures and cognitive deficits. Neuronal and dendritic losses along with remodeling of synaptic connectivity are pathological features often seen in hippocampal tissue resected from individuals with drug-resistant seizures and in experimental models of SE and epilepsy. However, the mechanisms underlying SE-induced remodeling of hippocampal synaptic circuitries are still elusive. Recent evidence points to the classical complement system as a candidate mechanism. The classical complement proteins C1q and C3b are associated with synaptic remodeling in the developing brain and have been shown to be altered in hippocampi from human and experimental epilepsy. Understanding the potential role that the classical complement pathway may play on the

dendritic and synaptic pathology associated with SE requires information on the extent to which C1q and C3b are altered and their distribution. Therefore, the objective of this study was to quantify SE-induced changes in the level and distribution of C1q and C3 proteins. SE was induced in with pilocarpine and stopped after 1 hour with diazepam. Hippocampi were collected at various time points after SE (3-, 14-, 21- and 35-days). Immunohistochemistry (IHC) and Western blots (WB) with antibodies against C1q and C3 along with densitometry analysis were used to identify and quantify subcellular and regional changes. IHC showed that C1q immunoreactivity was localized in the hippocampus of both control and SE rats. In control hippocampi, C1q signal was most evident within the stratum lacunosum moleculare while in SE hippocampi intense C1q signal was localized to the stratum radiatum. Densitometry analysis of immunoreactive signal showed a significant increase in the protein levels of C1q in the SE group compared to controls ($p<0.05$). C1q is the initiating protein of the classical pathway. Thus, an increase in C1q suggests increased activation after SE. WB showed a significant increase in the levels of C3 cleavage along with C3b fragments in the SE group compared to the control group ($p<0.05$). In order to act as an opsonin, C3 must be cleaved. This can occur downstream of C1q signaling. Therefore, an increase in C3 cleavage products further supports activation of at least the classical complement pathway after SE. Taken together these data suggest that SE triggers abnormal activation of the classical complement pathway in the hippocampus. Additional studies are underway to determine the role of this pathway in synaptic alterations associated with SE.

Disclosures: **N.D. Schartz:** None. **A.L. Brewster:** None.

Poster

688. Epilepsy: Glia and Post-Seizure Mechanisms

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Topic: B.11. Epilepsy

Title: Growth Associated Protein 43 (GAP-43) as a candidate biomarker of epileptogenesis

Authors: ***A. NEMES**[1], K. AYASOUFI[2], Z. YING[1], I. M. NAJM[1];
[1]Epilepsy Ctr., [2]Dept. of Immunol., Cleveland Clin., Cleveland, OH

Abstract: Epilepsy is a common, progressive neurological disorder affecting 1-2% of the population. Although various therapies are available, there is no known cure or biomarker of epilepsy. We have recently shown that growth associated protein 43 (GAP-43), a marker of axonal growth and synaptic plasticity, is highly and differentially expressed in tissue resected from patients with cortical dysplasia (CD), a malformation of cortical development associated with postnatal epileptogenesis. In the current study, we have found parallel results in a rat model

of CD following acute seizures. Baseline expression of GAP-43 was higher in CD rats as compared to controls. Acute seizures resulted in a significant and early increase of GAP-43 expression in both CD and control brains. However, GAP-43 expression continued to increase over time in CD, while returning to baseline in controls. Prolonged EEG monitoring revealed a progressive increase in the frequency of interictal epileptic spikes and the presence of spontaneous seizures in CD rats. Serum GAP-43 was significantly higher in CD rats with spontaneous seizures as compared to non-CD controls and non-epileptic CD rats. Double-labeling studies showed higher co-localization between GAP-43 positive axons and glutamatergic markers as compared to inhibitory markers, perhaps contributing to imbalanced neuronal networks leading to *in situ* small network hyperexcitation. Immuno-electron microscopic imaging supported this result with GAP-43 staining only found located at the growth cones of excitatory axons in a CD rat with spontaneous epilepsy. Finally, injection of a lentiviral vector containing siRNA against GAP-43 significantly decreased seizure duration and severity. Together, these results provide new insight into a potential candidate biomarker for CD and epileptogenesis; as well as a target for the treatment and prevention of progressive or intractable epilepsies.

Disclosures: **A. Nemes:** None. **K. Ayasoufi:** None. **Z. Ying:** None. **I.M. Najm:** None.

Poster

688. Epilepsy: Glia and Post-Seizure Mechanisms

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Topic: B.11. Epilepsy

Support: CURE Epilepsy

Title: Histological pathophysiology in models of postmalarial epilepsy

Authors: ***P. SSENTONGO**[1], A. ROBUCCIO[1], F. BAHARI[1], D. G. SIMS[1], J. W. BACCON[2], A. F. READ[1], S. J. SCHIFF[1], B. J. GLUCKMAN[1];
[1]CENTER FOR NEURAL ENGINEERING, PENNSYLVANIA STATE UNIVERSITY, UNIVERSITY PARK, PA; [2]Dept. of medicine, PENNSYLVANIA STATE UNIVERSITY, Hershey, PA

Abstract: Cerebral malaria (CM) annually affects more than 3 million children typically under the age of 5. CM carries a high mortality rate even when treated. Epilepsy occurs in 10-16 % of children who survive CM. We have developed the first murine model of post-cerebral malaria epilepsy (PoME). We report here on histological analysis from the cohort of animals studied for

the development of epilepsy, and correlate the neuropathophysiology with electrographic semiology.
Cohorts of mice of 3 different strain Swiss Webster (SW), CBA, and C57BL/6 were inoculated with Plasmodium *berghei* ANKA (PbANKA) and Plasmodium *berghei* NK65 (PbNK65) infected erythrocytes. After developing severe CM, they were treated with Artesunate and cured of the infection. They were then implanted with electrodes and chronically monitored with video/EEG for development of seizures. Littermate controls were inoculated with uninfected erythrocytes and treated identically to infected animals. EEG was analyzed to identify seizures, which were then scored for electrographic origin and evolution.
Following chronic recording, their brains were fixed, sectioned and stained with GFAP and NeuN for astrocytes and neurons. Sections were analyzed using stereological technique to quantify hippocampal volume and region-specific cell densities. These quantities along with peak infectious phase Parasitemia were then correlated with animal specific seizure semiology. Infected animals showed a range of morphological changes that included significantly expanded ventricles, damaged cortices, and asymmetrically affected hippocampi.
There is an urgent need of effective adjunct therapy during and following CM to mitigate the effects of the insult and prevent chronic irreversible brain damage and associated deficits. This murine model of PoME will provide a useful platform to test mechanistic approaches for such interventions.

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Poster

688. Epilepsy: Glia and Post-Seizure Mechanisms

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Topic: B.11. Epilepsy

Title: Energy metabolism activity in slices of dorsal and ventral hippocampus.

Authors: *A. I. IVANOV, G. BRANCATI, C. RAWAS, C. BERNARD;
INS, UMR1106, Marseille Cedex 05, France

Abstract: Differences in anatomical connectivity, gene expression patterns, and neuronal excitability in the dorsal and ventral regions of the hippocampus are well characterized. These differences account for the regional particularity of the neuronal network activity but do not explain why the ventral hippocampus is more prone to seizure genesis than the dorsal hippocampus. Since the ventral hippocampal network is more excitable than the dorsal therefore,

it can be proposed that the ventral hippocampus requires more energy supply. However, the regional specificity of energy metabolism in the hippocampus is not known.
Using local field potential recodings coupled either with NAD(P)H/FAD^+ imaging or with glucose, oxygen, and lactate sensing, we investigated metabolic activity induced by neuronal network stimulation in ventral and dorsal hippocampal slices of adult control mice and mice with epilepsy (pilocarpine model).
In control animals, we found that neuronal network activity induced by Schaffer collateral electrical stimulation was accompanied by similar decreases in tissue oxygen in ventral and dorsal slices, indicating equal level of the oxidative metabolism in both hippocampal regions. However glucose consumption was 2-5 times higher in ventral slices than in dorsal, indicating a high level of non-oxidative glucose utilization. The analysis of the NAD(P)H/FAD^+ fluorescence transients induced by the same neuronal network stimulation protocol showed no regional difference in the oxidative metabolism but revealed higher intensity of the cytosolic glycolysis in ventral than dorsal slices. Thus, to meet its high-energy demand, the ventral hippocampus network takes advantage of paralleled use of both oxidative and non-oxidative glucose metabolism pathways whereas for the dorsal network the yield of oxidative metabolism is sufficient.
We applied the same experimental paradigm in the experimental model of epilepsy. We found that the pathology erased a regional difference in metabolic signals mainly via a dramatic decrease of non-oxidative metabolism activity in ventral slices. All together our results suggest that i) there is a regional difference in energy metabolism activity; ii) to meet the energy demand required to fuel higher excitability, the ventral network takes advantage from parallel use of non-oxidative and oxidative metabolic path-ways; iii) in experimental epilepsy, non-oxidative cytosolic glycolysis is highly reduced leading to energy deficit that may contribute to the seizure genesis and/or propagation.

Disclosures: **A.I. Ivanov:** None. **G. Brancati:** None. **C. Rawas:** None. **C. Bernard:** None.

Poster

688. Epilepsy: Glia and Post-Seizure Mechanisms

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Program#/Poster#: 688.10/J9

Topic: B.11. Epilepsy

Title: Alzheimer's related disease pathologies following nerve agent-induced seizures.

Authors: *D. L. SPRIGGS, J. L. WINKLER, C. E. KAROLENKO, K. M. BOWENS, J. W. SKOVIRA;
USAMRICD, Gunpowder, MD

Abstract: Recent epidemiological and animal studies have suggested a link between organophosphate (OP) exposure and dementia related diseases such as Alzheimer's disease. Typically affecting individuals 65 and older, Alzheimer's disease causes progressive neurodegeneration and is the sixth leading cause of death in the US. Alzheimer's related pathologies are characterized by deposits of the protein fragment beta-amyloid (Aβ plaques) and abnormal twisting of strands of the protein tau (neurofibrillary tangles). Substantial evidence supports that aggregated Aβ and neurofibrillary tangles are sources of neurodegeneration. In this study we evaluated the progression of Alzheimer's related pathologies in Sprague Dawley rats after the onset of seizures induced by the organophosphorus nerve agent soman. Rats were pretreated with HI-6 (125 mg/kg, ip) 30 min prior to receiving soman (180 ug/kg, sc). This dose of soman is known to reliably elicit seizures in 100% of exposed animals. Atropine methylnitrate (2.0 mg/kg, im) was administered 1 min after soman exposure to increase survival. Control animals were given an equivalent volume of saline in place of a soman injection. Animals were euthanized 8 weeks following soman exposure. Our data show that the hallmarks of Alzheimer's related pathologies, beta-amyloid plaques and neurofibrillary tangles, are present in the cortex and hippocampus 8 weeks following exposure to the nerve agent soman. The rapid onset of Alzheimer's related pathologies following soman exposure highlight the need to investigate treatments to mitigate the development of these pathologies. If left untreated, OP casualties could face lifelong consequences and potentially early death from the development of these pathologies.
Disclaimer/Funding Statements. The experimental protocol was approved by the Animal Care and Use Committee at the United States Army Medical Research Institute of Chemical Defense and all procedures were conducted in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011), and the Animal Welfare Act of 1966 (P.L. 89-544), as amended.

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Poster

688. Epilepsy: Glia and Post-Seizure Mechanisms

Location: Halls B-H

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Program#/Poster#: 688.11/J10

Topic: B.11. Epilepsy

Title: Aberrant AMPA receptor and ADAR enzyme expression during the acute phase of pilocarpine-induced seizures increases neuronal death in the neonatal mouse brain

Authors: *S. JUNG, Y. BALLHEIMER, F. BRACKMANN, R. TROLLMANN;
Univ. Hosp. Erlangen, Erlangen, Germany

Abstract: Background: Neonatal seizure is a common pediatric emergency. During the acute seizure activity glutamate mediated excitotoxicity may contribute to aberrant apoptosis in the developing brain increasing the risk of survivors for developmental delay and later epilepsy. Since calcium-permeable α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors have important implication in disturbing glutamatergic transmission as well as early maturational glial and neuronal programs, we analyzed acute effects of pilocarpine-induced neonatal seizures on age- and region-specific cerebral expression of AMPA receptor subunits and AMPA receptor editing adenosine deaminases acting on RNA (ADAR).
Methods: Neonatal (P10) C57BL6/NCrl mice were injected intraperitoneally with methylscopolamine bromide (1 mg/kg, i.p.) and pilocarpine (400 mg/kg, i.p.). Seizures were terminated by diazepam (5 mg/kg, i.p.) 90 minutes after seizure onset. Thereafter, tissue preparation was performed after a regeneration period of 1 h, 24 h, and 72 h. Expression of AMPA subunits (GluR1, GluR2, GluR3, GluR4) and ADAR enzymes (ADAR1, ADAR2, ADAR3) was analyzed by real-time RT-PCR and immunohistochemistry. Induction of apoptosis-related gene expression (*bnip3*, *dusp1*, *ier3*) was monitored by real-time RT-PCR. Additionally, brain region specific apoptotic cell death was quantified by TUNEL and active caspase 3 staining.
Results: While absolute mRNA levels of AMPA receptor subunits GluR1, GluR2, GluR3, and GluR4 remained unaffected post pilocarpine-induced seizure activity, composition of the $GluR_{1/2/3/4}$ mRNA pool changed significantly within 1 h in pilocarpine-treated mice compared to controls. Thereby, the relative expression of GluR2 and GluR4 decreased significantly by 55% and 53%, respectively. While the mRNA level of regulatory ADAR3 remained stable over time, mRNA transcription of enzymatic active ADAR1 and ADAR2 enzymes was downregulated 24 h post pilocarpine treatment correlating with aberrant expression of BNIP3 and DUSP1 and increased TUNEL-positive cells in the subventricular zone.
Conclusions: Considering a crucial role of RNA-edited GluR2 for the permeability of AMPA receptors for calcium ions and subsequently calcium ions mediated neurotoxicity, aberrant expression of GluR2 as well as of the GluR2 pre-mRNA editing enzyme ADAR2 may explain enhanced and region specific activation of caspase 3 stimulated apoptosis. In conclusion, AMPA receptor antagonist may be a promising target to abolish excitotoxicity-induced neuronal loss in the developing brain.

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Poster

689. Epilepsy: Genetics and Genetic Models

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Topic: B.11. Epilepsy

Support: Dravet Syndrome Foundation Spain

Title: A novel inducible mouse model of Dravet syndrome

Authors: *M. SASNER[1], J. MORGAN[1], L. GOODWIN[1], K. CHENG[2], S. J. SUKOFF RIZZO[1], A. MINGORANCE[3];
[1]The Jackson Lab., Bar Harbor, ME; [2]Charles River Labs, Wilmington, MA; [3]Dravet Syndrome Fndn. Spain, Madrid, Spain

Abstract: Dravet syndrome (also called severe monoclinic epilepsy of infancy) is a rare, genetically determined disease with no effective therapies. Comorbidities include cognitive impairments, hyperactivity, attention deficits, delayed psychomotor development, sleep disorders, anxiety-like behaviors, impaired social interactions and premature death.
Existing Dravet mouse models are not ideal for therapy development projects for various reasons. Some have early onset lethality that prevents shipping and sharing of mice. Most are not widely available for drug testing efforts due to legal restrictions.
To address this need, we have developed a novel inducible model that is available for drug development projects with a no-cost license: B6(Cg)-*Scn1a*$^{tm1.1Dsf}$/J (#26133). Expression of the *Scn1a* gene is normal until exposed to *cre* activity, when the floxed wild-type exon 26 is deleted and an engineered exon 26 carrying the A1783V mutation (as found in human patients) is expressed. This means that un-induced mice are normal and healthy and can be easily distributed. As verified by sequencing of mRNA, mutant *Scn1a* expression can be induced in specific neuronal subsets by mating to the appropriate *cre*-expressing strain. The JAX mouse repository has wide variety of both broadly- and specifically-expressed *cre* lines. The engineered *Scn1a* allele is on a B6 genetic background. Initial experiments with mice generated using a ubiquitous *cre* on a C57BL/6J genetic background resulted in lethality at ~ 3 weeks of age consistent with reports from other Dravet mouse models. However, a significant percentage of mice generated using a ubiquitous *cre* on a 129S1 genetic background survive (preliminary results indicate that nutritional support promotes viability) and have potential to be used for evaluating novel therapies for Dravet syndrome.
We will present detailed characterization of both the seizure and comorbid neuropsychiatric phenotypes of this novel line. We expect that making this model and corresponding *cre*-expressing mice lines easily available will facilitate its use as a drug screening platform and the development of effective therapies for Dravet syndrome.

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Poster

689. Epilepsy: Genetics and Genetic Models

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Title: Investigation of doublecortin, GABA and V-GLUT immunoreactivties in the hippocampus of genetic absance epileptic rats: an ultrastructural study

Authors: *O. T. CILINGIR KAYA[1], D. GURSOY[1], C. MOORE[3], C. K. MESHUL[4,3], F. ONAT[2], S. SIRVANCI[1];
[1]Histology and Embryology, [2]Pharmacol. and Clin. Pharmacol., Marmara Univ. Sch. of Med., Istanbul, Turkey; [3]Veterans Hosp., Portland, OR; [4]Behavioral Neurosci. and Pathology, Oregon Hlth. and Sci. Univ., Portland, OR

Abstract: The subventricular zone of the lateral ventricles and the dentate gyrus (DG) of the hippocampus are two distinct areas of the brain in which neurogenesis occurs throughout the lifetime period. Recent studies report that there is a relation between neurological disorders and neurogenesis. It is generally accepted that imbalances between GABA-mediated inhibitory and glutamate-mediated excitatory neurotransmissions which induce spike and wave discharges play an important role in the pathogenesis of epilepsy. Genetic absence epilepsy rats from Strasbourg (GAERS), the animal model of absence epilepsy, is a strain in which hippocampal neurogenesis has not yet been investigated. Since the age of animals is one of the determinant features on neurogenesis, we used both 21-day- and 3-month-old rats from GAERS and Wistar control rats.
In the present study, we questioned whether newly born neural cells form synapses with GABAergic and glutamatergic structures and thus integrate into the local circuitry. For this purpose, brain tissues were obtained after intracardiac perfusion fixation and 300 μm-thick

coronal vibratome sections were collected. DG regions were dissected from the sections and processed for electron microscopic assessments. For immunocytochemical investigations, ultrathin sections were obtained by using an ultramicrotome and double-labeled either for doublecortin (DCX), as a marker of immature neurons, and GABA or DCX and vesicular glutamate transporter 1 (VGLUT-1) using postembedding immunogold method. Labeled sections were photographed on a transmission electron microscope. NIH Image Analysis (Image J) was used for the quantitative analysis. One-way anova and Tukey tests were used for the statistical analysis.
In all groups, DCX was seen co-localized with both GABA or VGLUT-1 in axon terminals, dendrites and somata. We observed that newly born DCX-positive neurons synapsed not only with GABAergic or glutamatergic profiles but also with DCX-positive cells. While DCX-positive profiles including GABA showed a tendency to increase in 21-day-old groups compared to 3-month-old groups; profiles double-labeled for DCX and VGLUT1 showed an increase in 3-month-old groups compared to 21-day-old groups. Besides, DCX labeling was significantly increased in profiles forming asymmetrical synapses compared to those forming symmetrical synapses.
In conclusion, our findings in the present study suggest that newly born DCX immunoreactive neurons synapse with GABAergic and glutamatergic neurons and thus contribute to the local hippocampal circuitry in absence epileptic immature and adult rats.

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Poster

689. Epilepsy: Genetics and Genetic Models

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Topic: B.11. Epilepsy

Support: U01NS090407

Title: Postictal respiratory dysfunction is the primary cause of Sudden Death in a Dravet mouse model

Authors: *E. BRAVO[1,2], Y. KIM[3], G. B. RICHERSON[4,2,5];
[2]Neurol., [3]Biomed. Engin., [4]Mol. Physiol. and Biophysics, [1]Univ. of Iowa, Iowa City, IA;
[5]VAMC, Iowa City, IA

Abstract: Sudden death is one of the most common causes of death in Dravet Syndrome (DS) patients with refractory epilepsy. The mechanism is still undefined. In a DS mouse model with an *Scn1a*$^{R1407X/+}$ mutation, death occurs after spontaneous or induced seizures. Experiments on DS mice have shown that postictal death after induced seizures is due to bradycardia and asystole [Kalume et al, 2013]. Postictal bradycardia and death can be prevented in DS mice with atropine, suggesting the mechanism involves excessive vagal tone. However, postictal breathing has not been measured during experiments in DS mice, so it is unknown if respiratory dysfunction contributes to postictal death. Here we studied mice with an *Scn1a*$^{R1407X/+}$ mutation to determine the role of respiratory dysfunction in postictal death after spontaneous and heat-induced seizures.Spontaneous and induced seizures were monitored in a mouse EMU while recording EEG, EMG, EKG, whole body plethysmography (breathing), body temperature, room temperature, and humidity. To induce seizures by hyperthermia, DS mice were exposed to a heat lamp to cause a continuous increase in body temperature to 43° C. To study death after spontaneous seizures, DS mice were continuously monitored in a mouse EMU for 24 hours per day until postictal sudden death spontaneously occurred. In a separate set of experiment mice was exposure to hypoxia in a plethysmography chamber changing gases from 21%O_2 to 100% N_2, while monitoring EKG and breathing.

When the last tonic spontaneous or induced seizures occurred, mice invariably had apnea followed by death. When terminal apnea occurred, EKG activity continued for 3-5 minutes with progressively worsening bradycardia followed by asystole. Death could be prevented after heat induced seizures by mechanical ventilation. Bradycardia and death could also be prevented with 1 mg/kg atropine, but at that dose seizures were not followed by apnea. Two days later, the same atropine dose did not prevent apnea, bradycardia or death. Atropine given at a dose of 0.03 mg/kg, which is sufficient to block peripheral muscarinic receptors, did not prevent apnea, bradycardia or death. Exposure of wild-type mice to anoxia led to progressive bradycardia that was not prevented by 1 mg/kg atropine.

We conclude that primary respiratory arrest can play an important role in death due to spontaneous and induced seizures in DS mice with a *Scn1a*$^{R1407X/+}$ mutation. Bradycardia and asystole were due to hypoxia resulting from the apnea, and not due to parasympathetic inhibition of the heart. These data may lead to an understanding of SUDEP mechanisms in DS patients, as well as shed light on possible therapeutic approaches to prevent SUDEP.

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Poster

689. Epilepsy: Genetics and Genetic Models

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Topic: B.11. Epilepsy

Support: NIH R01 NS333000

Title: West Syndrome-associated mutation GABRB3(N110D) produces spontaneous seizures and electroencephalogram abnormalities in mice.

Authors: *L. G. JACKSON[1], S. QU[2], V. JANVE[3], C. C. HERNANDEZ[2], R. L. MACDONALD[2];
[2]Neurol., [3]Neurosci. Grad. Program, [1]Vanderbilt Univ., Nashville, TN

Abstract: Epileptic encephalopathies are severe neurological disorders characterized by multiform and intractable seizures and cognitive and behavioral deficits. For example, West Syndrome patients have infantile spasms that generally begin in the first year and about one half of patients will have other seizure types including partial, myoclonic, tonic, and tonic-clonic seizures. EEGs have a hypsarrhythmia pattern. We have made a knock-in mouse model of a WS-associated mutation in the β3 subunit of the $GABA_A$ receptowr, *GABRB3(N110D)*. We have previously shown that *GABRB3(N110D)* produces abnormal GABA currents in HEK293T cells, however surface expression of mutant-containing receptors remains unchanged. This suggests that trafficking of mutant receptors is not impaired, but the receptors do not function properly. Animals expressing the mutation have spontaneous seizures, including tonic-clonic, myoclonic and tonic jerks, head and forelimb "jerks" followed by brief periods of immobility. Electroencephalographic recordings of *Gabrb3*$^{+/N110D}$ mice with video-synchronized EEGs for 24 hour periods has shown multiform changes. The characterization of this mouse model of an epileptic encephalopathy aims to uncover behavioral mechanisms underlying the disease in order to better identify alternative interventions for patients with WS.

Disclosures: L.G. Jackson: None. **S. Qu:** None. **V. Janve:** None. **C.C. Hernandez:** None. **R.L. Macdonald:** None.

Poster

689. Epilepsy: Genetics and Genetic Models

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 689.05/DP02 (Dynamic Poster)

Topic: B.11. Epilepsy

Support: R01 NS33300

Title: Mice harboring the Lennox-Gastaut syndrome associated GABRB3(D120N) mutation exhibit spontaneous seizures and electroencephalogram abnormalities.

Authors: *V. S. JANVE[1], S. QU[2], R. L. MACDONALD[2];
[1]The Grad. Program of Neurosci., [2]Neurol., Vanderbilt Univ., Nashville, TN

Abstract: The Lennox-Gastaut syndrome (LGS) is a rare but catastrophic childhood epilepsy with an early onset, multiple seizure types, a characteristic electroencephalogram (EEG) pattern, motor delays and neuropsychiatric disorders. Most patients have intractable seizures. The cause of LGS remains unknown for about a third of the patients, and currently there are no animal models to study the disease pathogenesis. We have generated a mouse harboring the LGS-associated *GABRB3(D120N)* mutation to address this need. Our *in vitro* studies demonstrated that the *GABRB3(D120N)* mutation markedly reduced GABA-evoked currents by reducing GABA potency but not the surface levels of $GABA_A$ receptors, altered single channel properties, and perturbed the GABA binding site (Janve et al. 2016). The mice with the *GABRB3(D120N)* mutation had multiple types of spontaneous seizures including typical absences, atypical absences (characteristic of LGS), head drops, Straub tail, and tonic seizures. Here we report the results from the synchronous electroencephalography (EEG)-electromyography (EMG)-video monitoring of freely moving mice (4-6 months old), carried out for ~24 hours. Our preliminary findings show that compared to C57Bl/6 control mice the mutant mice had significantly increased number of typical absence seizures (peak 4-6 Hz), and atypical absence seizures (peak 2-3 Hz) associated with behavioral arrest, especially during the light period (inactive time for mice). The typical and atypical absence seizures lasted for less than 10s and greater than 100s, respectively. Several head drop events were also accompanied during typical and atypical absence seizures. The mutant mice also had hundreds of rearing events during the dark period (active time for mice), along with repetitive movements in circles around the edge of the cage. These results indicate that the mice harboring the *GABRB3(D120N)* mutation could be a valuable model for studying LGS.

Disclosures: V.S. Janve: None. **S. Qu:** None. **R.L. Macdonald:** None.

Poster

689. Epilepsy: Genetics and Genetic Models

Location: Halls B-H

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Program#/Poster#: 689.06/J15

Topic: B.11. Epilepsy

Support: NIH R01NS333000

Title: GABRB3(D120N), a Lennox-Gastaut Syndrome-associated mutation, induces behavioral abnormalities in mice

Authors: *M. CATRON[1,2], S. QU[1], V. JANVE[1,2], C. C. HERNANDEZ[1], R. L. MACDONALD[3];
[2]Neurosci. Grad. Program, [3]Neurol., [1]Vanderbilt Univ., Nashville, TN

Abstract: Lennox-Gastaut Syndrome (LGS) is an epileptic encephalopathy, a severe form of epilepsy, which is often intractable and characterized by early childhood onset (age 1-8), an abnormal electroencephalogram (EEG), and a broad range of seizure types. Additionally, it is often accompanied by several comorbidities, including attention deficit hyperactive disorder, severe intellectual impairment, and other abnormal behaviors. A *de novo* mutation, *GABRB3*$^{+/D120N}$, was identified in a patient with LGS. The D120N mutation is in the β3 subunit of the γ-aminobutyric acid type-A receptor ($GABA_AR$), and is specifically located near the GABA binding pocket formed by this subunit. After initial *in vitro* evidence that D120N impairs $GABA_AR$ function, we generated a knock-in mouse containing the mutation. We hypothesized that this animal would not only have a similar seizure phenotype to the patient condition, but that the animal would have similar behavioral abnormalities as can be seen in both LGS patients and in the D120N patient specifically. The work presented here is a suite of behavioral tests aiming to compare the D120N mouse and human LGS. We have performed preliminary tests which have revealed abnormalities in anxiety, hyperactivity, and cognitive ability in the D120N mice. We hope that sufficient parallels may be drawn between the D120N mouse and LGS such that this animal might serve as a model for this disorder, allowing us to further investigate mechanisms underlying the disorder and potentially identify novel or modified treatment options for patients with LGS.

Disclosures: M. Catron: None. **S. Qu:** None. **V. Janve:** None. **C.C. Hernandez:** None. **R.L. Macdonald:** None.

Poster

689. Epilepsy: Genetics and Genetic Models

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Topic: B.11. Epilepsy

Support: NIH Grant NS072221

NIH/NIGMS IRACDA K12 GM000680

Title: Early life febrile seizures worsens epilepsy and behavioral phenotypes in adult GEFS+ mutants

Authors: *S. B. DUTTON[1,2], L. A. PAPALE[2], A. P. ESCAYG[2];
[1]Biology/ Neurosci. Program, Agnes Scott Col., Decatur, GA; [2]Emory Univ., Atlanta, GA

Abstract: Mutations in the voltage gated sodium channel (VGSC) gene *SCN1A*, which encodes the alpha subunit of the $Na_v1.1$ VGSC, are responsible for a number of epilepsy disorders including genetic epilepsy with febrile seizures plus (GEFS+) and Dravet Syndrome (DS). A common feature of patients with *SCN1A* mutations is the frequent occurrence of early-life febrile seizures (FSs). The presentation of FSs in this patient population is often more prolonged and severe than typically observed, raising the possibility that these early life events may influence epileptogenesis. To gain a better understanding of this relationship, we first investigated whether prolonged early-life FSs influence adult seizures phenotypes. To achieve this, we developed two paradigms; one modeling a single prolonged FS, and the other modeling repetitive FSs. Mice subjected to each were evaluated for seizure susceptibility and spontaneous seizure frequency at 2 - 7 months periods post FS induction. Of important clinical relevance, we found that early-life prolonged FSs reduces seizure thresholds and increases the frequency of spontaneous seizures during adulthood. In addition, the early-life FSs resulted in hyperactivity and impairments in social behavior and recognition memory during adulthood. Lastly, we determined the possible contribution of neuroinflammation to these behaviors by tracking expression of 84 genes 6-hrs after exposure to the early-life FSs paradigm. Findings from this study highlight the long-term negative impact of early-life FSs on disease outcomes in this patient population and the need for therapeutic interventions that could ameliorate disease progression.

Disclosures: S.B. Dutton: None. **L.A. Papale:** None. **A.P. Escayg:** None.

Poster

689. Epilepsy: Genetics and Genetic Models

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Program#/Poster#: 689.08/J17

Topic: B.11. Epilepsy

Support: Seattle Children's Research Institute Seed Funds

NIH

Title: Cas9-targeted KCNQ5 KO creates a mouse model for late-onset epilepsy

Authors: *A. D. WEI[1], F. KALUME[1,2], A. M. BARD[1], N. SAHAI[1], P. WAKENIGHT[1], T. ZWINGMAN[1], W. DOBYNS[1,3], K. MILLEN[1,3], J.-M. RAMIREZ[1,2];

[1]Seattle Children's Res. Inst., Seattle, WA; [2]Dept. of Neurolog. Surgery, [3]Dept. of Pediatrics, Univ. of Washington, Seattle, WA

Abstract: The mammalian KCNQ (Kv7) gene family encodes voltage-gated K^+ channel subunits that assemble in homomeric and heteromeric combinations to conduct M-currents, in association with a family of single-transmembrane accessory subunits (KCNE1-5). Four of the 5 members of the KCNQ gene family are linked to human hereditary channelopathies affecting cardiac, brain and auditory function, with the conspicuous exception of KCNQ5. Amongst the KCNQ genes, KCNQ5 uniquely expresses in both CNS and muscles, including vascular smooth muscles. We now report a mouse model for late-onset epilepsy caused by Cas9-targeted knockout of KCNQ5. Cas9/sgRNA was constructed to target a PAM site in exon 5 which encodes the extracellular turret domain 5' of the P-selective filter. Hybrid Black Swiss-C57BL/6J ES cells were transfected, and one ES clone carrying two independent biallelic deletions was selected for blastocyst injections. These targeted deletions resulted in frameshift mutations truncating the predicted channel protein within the turret domain. Both deletions thus likely result in loss of function selective for KCNQ5 homomeric channels, in contrast to a previously created dominant-negative KCNQ5 mouse line (T. Jentsch Lab) which may also inactivate heteromeric channels, since heteromerization association domains encoded in the cytosolic C-terminal domain are absent in our lines. Founder mice were recovered and bred to create homozygous lines for each of the two KCNQ5 deletional KOs. Homozygous KCNQ5 KOs are viable, but exhibit reduced fecundity and overt behavioral seizures beginning at mature adult ages (>P150). Seizures were observed with mild stressors such as cage changing, and manifested as both tonic clonic convulsions and absence-type behavioral arrests. Extended video/cortical EEG recordings from mature KCNQ5 KO animals revealed abnormal baseline high frequency activity and numerous interictal spikes. Propensity for abnormal EEG activities was increased following acute sleep deprivation. KCNQ5 KO animals also exhibited thermal seizures when their body temperatures were elevated to 40^o, indicative of susceptibility to febrile seizures. In addition, younger adults (~P50) were hyperactive by open-field assays. Taken together, these observations suggest that KCNQ5 is a significant new mammalian susceptibility gene for epilepsy.

Disclosures: **A.D. Wei:** None. **F. Kalume:** None. **A.M. Bard:** None. **N. Sahai:** None. **P. Wakenight:** None. **T. Zwingman:** None. **W. Dobyns:** None. **K. Millen:** None. **J. Ramirez:** None.

Poster

689. Epilepsy: Genetics and Genetic Models

Location: Halls B-H

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Program#/Poster#: 689.09/J18

Topic: B.11. Epilepsy

Title: Rescue of epileptic phenotype in Synapsin I knockout mice by inhibiting eEF2K/eEF2 pathway

Authors: *C. SALA[1], L. PONZONI[2], C. HEISE[1], E. TAHA[3], L. GRITTI[1], F. C. GUARNIER[4], A. MOSSA[1], C. MONTANI[1], P. SCALMANI[5], M. MANTEGAZZA[6], F. VALTORTA[4], M. SALA[1], C. VERPELLI[1], K. ROSENBLUM[3];
[1]CNR Neurosci. Inst., Milano, Italy; [2]BIOMETRA, Univ. degli Studi di Milano, Milano, Italy; [3]agol Dept. of Neurobiology, Univ. of Haifa, Haifa, Israel; [4]Div. of Neuroscience, San Raffaele Scientific Inst. and Vita-Salute Univ., Milano, Italy; [5]U.O. of Neurophysiopathology and Diagnos. Epileptology, IRCCS Neurolog. Inst. Carlo Besta,, Milano, Italy; [6]Inst. of Mol. and Cell. Pharmacol. (IPMC), CNRS UMR7275 and Univ. of Nice-Sophia Antipolis, Valbonne, France

Abstract: Several mutations causally linked to epilepsy have been identified in genes coding for ion channels and/or involved in neuronal development and synaptic transmission. An array of mutations in the Syn genes in human has been associated with epilepsy and/or autism spectrum disorders, diseases that display a frequent comorbidity. Although a plethora of drugs for the treatment of epilepsy are available, the disease cannot be adequately controlled in a relevant percentage of patients. Here, we report that the eEF2K/eEF2 pathway is essential for regulating excitation/inhibition balance in hippocampal circuits. We found that loss of eEF2K increased GABAergic synaptic transmission while up-regulating a defined subset of synaptic proteins. Because pharmacological or genetic inhibition of eEF2K can revert susceptibility to seizure in the Synapsin I knockout mice, we suggest that eEF2K is a potential novel target for antiepileptic drugs.

Disclosures: C. Sala: None. **L. Ponzoni:** None. **C. Heise:** None. **E. Taha:** None. **L. Gritti:** None. **F.C. Guarnier:** None. **A. Mossa:** None. **C. Montani:** None. **P. Scalmani:** None. **M. Mantegazza:** None. **F. Valtorta:** None. **M. Sala:** None. **C. Verpelli:** None. **K. Rosenblum:** None.

Poster

689. Epilepsy: Genetics and Genetic Models

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Topic: B.11. Epilepsy

Support: Epilepsy Foundation/AES

Title: Neuropathology of Munc18-1 mutants underlying infantile epilepsies

Authors: ***N. G. L. GUIBERSON**[1], A. PINEDA[1], K. E. CARNAZZA[1], J. S. DITTMAN[2], J. BURRÉ[1];
[1]Brain and Mind Res. Inst., [2]Dept. of Biochem., Weill Cornell Med., New York, NY

Abstract: Munc18-1 controls neurotransmitter release by regulating the SNARE protein syntaxin-1. Mutations in Munc18-1 are associated with Ohtahara, West, and Dravet syndromes, three devastating infantile epileptic encephalopathies. However, the mechanistic relationship between Munc18-1 mutations and these diseases is unknown. We aim to determine how mutations in Munc18-1 cause neuronal dysfunction. We hypothesize that these disease-related mutations cause defects in its folding, stability and localization, which subsequently impair syntaxin-1 and synapse function. Stability and turnover of Munc18-1 mutants were analyzed in transfected N2a cells via cycloheximide chase experiments. Solubility profiling, limited proteolysis, and immunocytochemistry were used to assess aggregation of Munc18-1 mutants. Syntaxin-1 stability, turnover, aggregation, and subcellular localization were assessed by the same paradigms used for Munc18-1. We found that Munc18-1 mutants are unstable and are turned over faster than wild-type Munc18-1. Mutants also demonstrated aggregation and decreased solubility. The reduction in Munc18-1 levels triggered a corresponding decrease in syntaxin-1 levels and stability, suggesting a loss-of-function disease mechanism of both Munc18-1 and syntaxin-1. To analyze the effect of Munc18-1 mutants on synapse function *in vivo*, we generated transgenic *C. elegans* strains overexpressing wild-type unc18 or the G544D mutant on an unc18 null background, and quantified the ability of these overexpressions to rescue the unc18 null phenotype by locomotion, heat shock, and aldicarb assays. Wild-type unc18 but not the G544D mutant ameliorated the synaptic defects in unc18 null worms. GFP-tagged wild-type unc18 showed diffuse somatic and axonal distribution while the G544D mutant aggregated. Overall, this work provides insight into the basic pathobiology of mutant Munc18-1 by demonstrating its instability, tendency to aggregate and impaired interaction with syntaxin-1 *in vitro*, as well as its profound mislocalization and failure to rescue synaptic function *in vivo*.

Disclosures: N.G.L. Guiberson: None. **A. Pineda:** None. **K.E. Carnazza:** None. **J.S. Dittman:** None. **J. Burré:** None.

Poster

689. Epilepsy: Genetics and Genetic Models

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Topic: B.11. Epilepsy

Support: Alberta Prion Research Institute Grant 201300010

NSERC Grant RGPIN/03819-2014

CIHR Grant MOP-130495

Title: Repeated seizures drive hippocampal amyloid plaque deposition and memory impairment in a transgenic mouse model of Alzheimer's disease

Authors: *J. S. SPARLING, J. S. FARRELL, K. ADDO-OSAFO, G. C. TESKEY, P. K. STYS;
Hotchkiss Brain Institute, Univ. of Calgary, Calgary, AB, Canada

Abstract: Chronic temporal lobe epilepsy (TLE) involves prominent and progressive sclerosis and atrophy of the mesial temporal lobe which is thought to contribute to epileptogenesis. Given that amyloid-β (Aβ) is synaptically released in an activity-dependent manner, we hypothesized that seizures promote the generation and accumulation of β-amyloid. These deposits of potentially toxic Aβ aggregates might establish foci of neurodegeneration in the temporal lobe, leading to sclerosis and degeneration in these key areas, and thus contributing to the pathogenesis of TLE. To enhance the deposition of Aβ released by excessive electrical activity, here we chose to study the effect of seizures in 5XFAD transgenic mice, which harbor transgenes for five human familial AD (presenilin and amyloid precursor protein) mutations. Mice were implanted with unilateral hippocampal electrodes and received no stimulation (sham group; n = 14) or 60Hz kindling stimulation to elicit 21 hippocampal seizures (seizure group; n = 14) between the ages of P30 and P60 prior to perfusion and tissue collection at P61. Because we previously found that hypoxia occurs after seizure induction, and that this effect is COX-2 dependent and can be prevented with acetaminophen, we also treated half of the mice in each group with acetaminophen to examine the role of concomitant hypoxia in Aβ deposition. Amyloid deposition was assessed microscopically using tissue sections stained with the fluorescent amyloid probe X-34. **Results**: Seizure duration and severity (Racine scale) did not differ between vehicle- and acetaminophen-treated mice. Sham (seizure-free) control mice showed no amyloid deposition in the hippocampi at P61. In contrast, mice subjected to unilateral stimulation exhibited marked bilateral amyloid deposition in both hippocampi, and novel object recognition testing revealed significant memory impairment in this group. Acetaminophen prevented hippocampal hypoxia and memory impairment, and partially reduced amyloid deposition, indicating a significant contribution of hypoxia to the seizure-induced hippocampal amyloidosis. **Conclusion**: Seizure-related electrical hyperactivity promotes significant hippocampal amyloid deposition in the 5XFAD transgenic mouse. While this strain was deliberately chosen to exaggerate plaque generation, reports of AD-like amyloid pathology in human TLE raise the possibility that chronic (possibly subclinical) seizure activity promotes potentially toxic amyloid deposition that contributes to the pathogenesis of TLE.

Disclosures: J.S. Sparling: None. **J.S. Farrell:** None. **K. Addo-Osafo:** None. **G.C. Teskey:** None. **P.K. Stys:** None.

Poster

689. Epilepsy: Genetics and Genetic Models

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Topic: B.11. Epilepsy

Support: NIH Grant R01 NS082343 to PBC

CURE award 30018775 to PBC

Title: mTOR-dependent changes in cellular morphology following GATOR complex subunit knockdown: novel insights into DEPDC5 and NPRL3 associated cortical malformation

Authors: *P. H. IFFLAND, M. BAYBIS, P. B. CRINO;
Lewis Katz Sch. of Med. Temple Univ., Philadelphia, PA

Abstract: Malformations of cortical development (MCD) are common causes of neurological deficits in children including seizures and intellectual disability. Recent studies have found loss-of-function mutations in genes encoding the DEPDC5 and NPRL3 subunits of the mTOR regulatory complex GATOR 1. These mutations have been linked to various MCD-subtypes associated with seizures. However, the cellular consequences of these mutations remain undefined. We hypothesize that knockdown (KD) of DEPDC5 or NPRL3 leads to enhanced cell size, altered cell polarity, and altered subcellular localization of mTOR.
Multiple cells lines, including HEK 293FT, N2A and mouse neural progenitor cells (mNPCs) were used to define the morphological consequences of shRNA KD of DEPDC5 or NPRL3 compared to control scramble shRNA construct. Cell size was assessed by FACS or direct measurement of the soma in digital images. Outgrowth of actin labeled filopodia in mNPCs was assessed in digital images. The mTOR dependency of DEPDC5 or NPRL3 KD was determined using rapamycin (50 nM, 48hrs) or a p70S6 kinase inhibitor (PF-4708671; 1nM, 48 hrs.). Functional localization of mTOR to the lysosomal membrane or cytoplasm following DEPDC5 or NPRL3 KD was determined by immunochemical staining and colocalization analysis examining the proximity of mTOR to the lysosomal surface (LAMP 2 staining) with or without rapamycin or PF-4708671 treatment.
HEK 293FT and N2A cells transfected with DEPDC5 or NPRL3 shRNA were larger than scramble control or wildtype cells based on FACS analysis (n= 100,000 cells per group). Direct measurements of mNPCs revealed a larger soma size in DEPDC5 or NPRL3 KD cells compared to scramble control (n= 50; $p < 0.05$). mNPCs transfected with DEPDC5 or NPRL3 shRNAs displayed an increased number of filopodia versus scramble control (n= 50 per group; $p < 0.05$). DEPDC5 or NPRL3 transfected cells treated with rapamycin or PF-4708671 were significantly smaller in size as measured by both FACS (N2A and HEK 293FT; n= 100,000) and direct measurement (mNPCs, n= 50). There was a higher degree of mTOR and lysosomal protein

colocalization in KD versus rapamycin treated cells following DEPDC5 or NPRL3 KD in mNPCs. As PF-4708671 acts downstream of mTORC1, no change was observed in colocalization in treated cells.
We demonstrate changes in cellular morphology in multiple cell lines after DEPDC5 or NPRL3 KD. These findings recapitulate what is observed in patients with DEPDC5 or NPRL3 mutations and a rat model of DEPDC5 knockout. Further, we have defined the mTOR–dependency of these morphological abnormalities, which may provide novel avenues for treatment of MCDs associated with GATOR complex subunits.

Disclosures: P.H. Iffland: None. **M. Baybis:** None. **P.B. Crino:** None.

Poster

689. Epilepsy: Genetics and Genetic Models

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Topic: B.11. Epilepsy

Support: RO1 NS082761-02

Title: Network dysfunction in Arx expanded mice; developing an insight into a pediatric epileptic network.

Authors: *F. SIDDIQI, A. MCCOY, L. OLEAR, D. JOSEPH, E. MARSH;
Dept. of Neurol., Children's Hosp. of Philadelphia, Philadelphia, PA

Abstract: ARX is a transcription factor expressed throughout interneuron development. Mutations in ARX lead to a range of neurological disorders with epilepsy being common to all cases. Little is known about why children with ARX mutations have seizures and developmental delays. To address this question, we have utilized an Arx mutant mouse (Arx^{E}) that recapitulates some of the human phenotypes. We previously demonstrated aberrations within hippocampal interneurons and pyramidal cells as well as local CA1 network dysfunction. To further probe the network level dysfunction in Arx^{E} mice we recording neuronal activity using in-situ multi electrode array (MEA) and in vivo multi-unit recordings.
Post-natal day (P)30 to P52,Arx^{E} mice and age matched WT littermates, both on a Bl6 background were employed. For MEA recordings, 350 µm Hippocampal-entorhinal cortical slices were prepared using Leica Vibratome in aCSF with 192mM sucrose and then incubated at 37°C in a water bath for 45 min and then equilibrated to room temp for 1 hr in recording aCSF. The slices were then transferred to the MEA (Multi Channel Systems) recording system that spans the hippocampus. 15 min of baseline gap-free data was collected, followed by an IO

stimulus protocol and finally a repetitive stimulus protocol (2s, 60Hz train, 100μs pulse width with 10min intervals repeated 3x's). For in vivo recordings in house made 25um tetrode bundles were placed into CA1, CA3 regions in the hippocampus using a microdrive. Unit activity was recorded and analyzed using DataWave Software. Finally, immunohistochemistry was performed using NeuN and Delta FosB.
MEA recordings demonstrated increased unit firing and ictal discharges in Arx^{E} mice in the CA3 region compared to controls while CA1 is hypo-excitable, with reduced unit firing and fewer ictal discharges. Further analysis of I/O curves is pending. In vivo multi-unit recording was performed to determine if the changes in network level activity in the slice are present in vivo. Lastly, our histological studies demonstrate no changes in neuronal numbers in CA1 (NueN staining) with paradoxical hyperactivation of CA1 compared to CA3 as determined by an increase in Delta FosB activity.
First, we confirm our previous studies showing hypoexcitability in CA1 using a different approach to record network activity. The MEA results demonstrate local network dysfunction, which is also confirmed with delta FosB staining. The dynamic alterations in activity in CA3 and CA1 lead to the epileptic phenotype in these Arx^{E} mice. By highlighting regions of dysfunction in the ARX mice we can begin to consider targeted approaches to restoring normal function.

Disclosures: F. Siddiqi: None. **A. McCoy:** None. **L. Olear:** None. **D. Joseph:** None. **E. Marsh:** None.

Poster

689. Epilepsy: Genetics and Genetic Models

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Topic: B.11. Epilepsy

Support: NS078184

Title: A de novo mutation in KCNT2 (SLICK) gene produces a change of channel function and underlies an epileptic encephalopathy phenotype

Authors: *S. GURURAJ[1], E. PALMER[3], G. SHEEHAN[1], T. KANDULA[3], R. MACINTOSH[3], W. LO[4], G. ELAKIS[4], Y. ZHU[5], M. BUCKLEY[4], M. DINGER[6], M. COWLEY[6], R. SACHDEV[3], E. KIRK[3], T. ROSCIOLI[3], A. BYE[3], M. E. DUFFEY[1], A. BHATTACHARJEE[2]; [2]Pharmacol. and Toxicology, [1]Univ. at Buffalo - The State Univ. of New York, Buffalo, NY; [3]Sydney Children's Hosp., Sydney, Australia; [4]SEALS Pathology, Randwick, Australia; [5]Genet. of Learning Disability Service, Waratah, Australia; [6]Garvan Inst., Sydney, Australia

Abstract: There are two members of the sodium-activated potassium (K_{Na}) channel family- KCNT1 (SLACK) and KCNT2 (SLICK), which exhibit similarities in sequence and structure yet displaying distinct expression patterns, function and regulation throughout the central and peripheral nervous systems. Several mutations of the KCNT1 gene have been recently described as causal to epileptic disorders, almost universally associated with cognitive impairments. Predominantly, these variants have been shown to be gain-of-function mutations. We report the first identification of a KCNT2 channel variant associated with an epileptic encephalopathy phenotype. We have identified a *de novo* heterozygous missense mutation at Phe240Leu in the pore region of KCNT2 in a patient with severe epileptic encephalopathy. Electrophysiological studies were performed in recombinant mammalian cell systems and in *Xenopus Laevis* oocytes to characterize the phenotypic consequences of this mutation to understand its role in pathogenicity. First, the Phe240Leu mutation appears to behave as a gain-of-function mutation, wherein the Phe240Leu KCNT2 channels exhibit significantly higher currents compared to Wild Type (WT) KCNT2, in the case of both rat Kcnt2 channels recorded in CHO cells and human KCNT2 channels recorded in *Xenopus* oocytes. We demonstrate that the gain-of-function is in fact the result of constitutive activation of channels. Second and more significantly, the Phe240Leu mutation appears to produce a 'change-of-function' in the channel, in that mutant KCNT2 channels demonstrate a pronounced rightward shift in reversal potential (towards E_{Na}) as would be the case for non-selective cation channels. These indicate that the Phe240Leu mutation results in a sodium-permissive conductance compared to predominantly potassium-selective WT KCNT2 conductances. Examining surface expression of the channels in *Xenopus* oocytes using membrane biotinylation assays revealed that Phe240Leu KCNT2 channels are significantly lower at the membrane as compared to WT channels, suggesting that mutated channels may be subject to misfolding and are targeted for degradation or undergo reduced trafficking to the membrane. Taken together, our results identify a change-of-function Phe240Leu *KCNT2* mutation to be the first description of altered selectivity of KCNT2 channels, and indeed of K_{Na} channels. Of clinical importance, the above described findings are evidence to establish pathogenicity of the Phe240Leu Slick mutation in the reported epileptic encephalopathy patient.

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Poster

689. Epilepsy: Genetics and Genetic Models

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103-2628-B-010-002-MY3

104-2633-H-010-001

105-2633-B-009-003

NHRI-EX103-10314NC

Title: Loss-of-function effects of PRRT2 mutations lead to neuronal dysfunction and neurodevelopmental defects

Authors: *F. NIAN[1,2], Y.-T. LIU[3,4], W.-J. CHOU[5], C.-Y. TAI[8], S.-Y. KWAN[3,4], C. CHEN[3,4], P.-W. KUO[5], P.-H. LIN[4], C.-Y. CHEN[9], C.-W. HUANG[5], Y.-C. LEE[3,4,6], B.-W. SOONG[3,4,6], J.-W. TSAI[5,6,7];
[1]Inst. of Brain Sci., Sch. of Med., Taipei, Taiwan; [2]Program in Mol. Med., Natl. Yang-Ming Univ. and Academia Sinica, Taipei, Taiwan; [3]Dept. of Neurology, Neurolog. Inst., Taipei Veterans Gen. Hosp., Taipei, Taiwan; [4]Dept. of Neurol., Natl. Yang-Ming Univ. Sch. of Med., Taipei, Taiwan; [5]Inst. of Brain Sci., [6]Brain Res. Ctr., [7]Biophotonics and Mol. Imaging Res. Ctr., Natl. Yang-Ming Univ., Taipei, Taiwan; [8]Istitute of Pharmaceutics, Develop. Ctr. for Biotech., New Taipei City, Taiwan; [9]Grad. Inst. of Life Sci., Natl. Def. Med. Ctr., Taipei, Taiwan

Abstract: Mutations in *PRRT2* (proline-rich transmembrane protein 2) cause a wide spectrum of neurological diseases, ranging from paroxysmal dyskinesia to epilepsy and mental retardation. Previous *in vitro* studies suggested that PRRT2 interacts with SNAP25 (synaptosomal-associated protein, 25kD) and may be involved in neurotransmitter release at the synapses. However, it is still not fully understood how mutant PRRT2 causes pathological effects on the nervous system *in vivo*. Using synaptic membrane fractionation and immunostaining techniques, we first showed that Prrt2 was localized at the pre- and post-synaptic membranes with a close spatial association with SNAP25 in rat neurons. We then examined the subcellular localization of six truncating *PRRT2* mutations identified in our Taiwanese patients of paroxysmal kinesigenic dyskinesia (PKD) and found that these mutant proteins accumulated in the cytoplasm and thus failed to target to the cell membrane. We also found that the R308C missense mutant protein had significantly reduced expression regardless of its correct localization at the plasma membrane. These results suggested loss-of-function effects generated by these mutations. To test the pathological effects of PRRT2 loss-of-function *in vivo*, we used *in utero* electroporation of Prrt2 shRNA to knock down Prrt2 expression in the neocortex of mouse embryos. Surprisingly, Prrt2 knockdown in cortical neurons led to delayed neuronal migration, demonstrating a novel role of PRRT2 in embryonic neural development. We further utilized two-photon microscopy to investigate the effects of Prrt2 knockdown on synapses of GFP-labeled neurons *in vivo* and

found a marked decrease in the density of dendritic spines in the brain at adolescence. In conclusion, our study using advanced *in vivo* techniques uncovered a novel mechanism whereby PRRT2 loss-of-function caused by altered subcellular localization and/or decreased expression leads to abnormal neuronal migration and synaptic development. These defects in turn results in the severe neurological symptoms in *PRRT2*-related diseases.

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Poster

689. Epilepsy: Genetics and Genetic Models

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Title: Age-dependent plasticity of cortical GABAergic innervation lessens seizure severity in Cacna1a conditional mutant mice

Authors: *X. JIANG;
neuroscience, CHU Res. Centre, Univ. of Montreal, Montreal, QC, Canada

Abstract: *CACNA1A* loss-of-function mutations result in cerebellar ataxia and epilepsy in humans. We previously showed that the pre-natal deletion of *Cacna1a* in the $Nkx2.1^{Cre}$; $Cacna1a^{c/c}$ mutant mice, causing the ablation of voltage-gated $Ca_V2.1$ Ca^{2+} channels in forebrain GABAergic interneurons (IN), results in synaptic impairment of parvalbumin (PV) fast-spiking basket cells and induces generalized epilepsy. As *CACNA1A* mutation-associated epilepsy improves with age in patients, we propose that specific compensatory mechanisms may occur that, in the face of cortical disinhibition, re-establish the inhibition/excitation balance with time. We generated conditional mutant mice carrying a post-natal deletion of *Cacna1a* in PV^+ neuronal populations (PV^{Cre};$Cacna1a^{c/c}$). PV^{Cre};$Cacna1a^{c/c}$ mutant mice develop cerebellar ataxia and a mild epileptic phenotype with spike-wave seizures after postnatal day 45 (P45). We show a comparable reduction of GABAergic perisomatic boutons on cortical PC and a similar impairment of synaptic release from PV-INs in paired-recordings in both pre-natal ($Nkx2.1^{Cre}$)

and post-natal (PV^{Cre}) mutants, suggesting that both mutant lines develop a significant impairment of perisomatic inhibition. However, surprisingly, $PV^{Cre};Cacna1a^{c/c}$ mutants displayed a two-fold increase in the frequency of miniature inhibitory synaptic currents (mIPSC) in cortical pyramidal cells (PC) at P60, whereas these events were unchanged in $Nkx2.1^{Cre};Cacna1a^{c/c}$ mutants at P20, suggesting that age-dependent compensatory plasticity changes in GABAergic circuits occur in the $PV^{Cre};Cacna1a^{c/c}$ mutant mice. In particular, we demonstrate a significant increase of functional dendrite-targeting GABAergic projections from somatostatin (SOM) INs in $PV^{Cre};Cacna1a^{c/c}$ mutants, using a combination of paired-recordings, immunostaining and two-photon imaging. Therefore, we propose that, in the face of altered PV-INs synaptic function, progressive reorganization of dendritic inhibition, including synaptic connectivity of SOM-INs, restricts cortical excitability and lessens seizure severity in $PV^{Cre};Cacna1a^{c/c}$ mutants. A similar phenomenon has recently been described in chronic post-status epilepticus epilepsy models suggesting that this is a common phenomenon in genetic and non-genetic forms of chronic epilepsy.

Disclosures: X. Jiang: None.

Poster

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Topic: B.11. Epilepsy

Support: NIH 5UM1HG007301-03

Title: A pipeline for rapid *In vitro* evaluation of variants of uncertain significance from patients with developmental delay and/or intellectual disability exhibiting seizures

Authors: *J. COCHRAN[1], K. L. ENGEL[1], A. A. HARDIGAN[1], M. D. AMARAL[1], K. M. BOWLING[2], C. R. FINNILA[2], S. M. HIATT[2], M. L. THOMPSON[2], D. E. GRAY[2], J. S. WHITTLE[2], W. V. KELLEY[3], M. E. COCHRAN[3], K. M. EAST[3], N. E. LAMB[3], C. A. RICH[5], K. B. BROTHERS[5], E. J. LOSE[6], S. B. SIMMONS[7], E. M. BEBIN[7], G. S. BARSH[4], G. M. COOPER[2], R. M. MYERS[1];
[1]Myers Lab., [2]Gregory Cooper Lab., [3]Educ. & Outreach, [4]Barsh Lab., Hudsonalpha Inst. For Biotech., Huntsville, AL; [5]Pediatrics, Univ. of Louisville, Louisville, KY; [6]Genet., [7]Neurol., Univ. of Alabama at Birmingham, Birmingham, AL

Abstract: Developmental delay and intellectual disabilities (DD/ID) include devastating phenotypes and comprise a large fraction of rare undiagnosed conditions in children.

Unfortunately, little is known about the cellular mechanisms that lead to disease, and as a consequence, therapeutic advancements have suffered. Previous studies have indicated a strong genetic component to these phenotypes, and successful identification of causal genetic variants through exome or whole genome sequencing sometimes leads to clinical diagnoses, revision of treatment strategies, community and support network building, and increased quality of life. However, sequencing-based diagnostic efforts typically solve only a subset of cases; while precise numbers vary according to ascertainment and study enrollment criteria, large fractions of children cannot be given a precise genetic diagnosis even after whole genome sequencing. In our own work as part of the Clinical Sequencing Exploratory Research consortium, we have found diagnostic genetic variants in ~27% of children with conditions refractory to standard diagnostic tests. Rigorous experimental evaluation of variants and genes implicated in DD/ID is needed to increase diagnostic rate, particularly for the subset of the undiagnosed cases where a potential genetic cause is identified but not confirmed, termed to be variants of uncertain significance (VUSs). VUSs typically arise as a result of a lack of information about the relevance (or lack thereof) of a given gene to disease, the impact (or lack thereof) of a given variant on gene function, or both. In our studies to date, ~15% of affected children harbor a VUS, and others harbor variants that may be of interest, but are non-returnable even as a VUS. We are developing a pipeline to assess the effect of VUSs and non-returnable variants in human neurons derived from neural precursor cells. We have tailored the design of this pipeline to detect changes in excitability, as ~50% of our patient population exhibits seizures. This pipeline will provide evidence for or against the association of these sequence variants on key molecular and cellular phenotypes including global gene expression, neuronal excitability, and synapse and/or ion channel composition profile. Thus, this pipeline will provide critical insights regarding the biological roles of genes and variants associated with DD/ID and, more broadly, will establish a framework for future mechanistic interrogation of genetic variation as it relates to other neurologic diseases.

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Poster

689. Epilepsy: Genetics and Genetic Models

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Topic: B.11. Epilepsy

Support: NIH Grant NS031348

Title: Tmem151b, a novel seizure susceptiblity gene encoding a putative ER protein

Authors: *W. N. FRANKEL[1], T. C. MCGARR[2], S. SUKOFF-RIZZO[2], R. M. BOUMIL[2], S. PETRI[1];
[1]Columbia Univ. Med. Ctr., New York, NY; [2]The Jackson Lab., Bar Harbor, ME

Abstract: A new mouse mutant - Nmf389 - was identified in the former Neuroscience Mutagenesis Facility (NMF) of The Jackson Laboratory. Homozygous adults have a significantly reduced electroconvulsive threshold, often bypassing the minimal generalized seizure endpoint to maximal tonic-clonic seizures at low stimulus settings. Heterozygotes have a modestly reduced electroconvulsive threshold, but are not especially susceptible to pentylenetetrazole- or kainate-induced tonic-clonic seizures, although some homozygotes have been observed to exhibit handling-associated tonic-clonic seizures later in life. The recessive electroconvulsive phenotype was mapped to Chr 17, and whole brain RNA sequencing was used to identify a single nucleotide mutation in the Tmem151b gene, encoding an anonymous, putative 2-transmembrane domain containing protein of unknown function. The Nmf389 mutation created a new splice acceptor site 42 nt in exon 3, used by all Tmem151b mRNA, corresponding to an in-frame deletion of 14 amino acids. Confirming candidacy, the Tmem151b knockout allele (from the NIH KOMP program) conferred a similarly low seizure threshold and did not complement the low seizure threshold of Nmf389. While further phenotypic assessment of knockout homozygotes identified no interictal EEG abnormalities or other overt phenotypes, comprehensive behavior profiling revealed homozygotes to be more hyperactive than wildtype littermates with a suggestion of anxiolytic-like behavior. Grossly the brain histology was normal, except for patchy H&E staining in the molecular areas of the hippocampus, between CA1 and dentate gyrus. Confocal imaging of Tmem151b homozygous, YFP-H transgenic mice revealed abnormal dendritic branching, currently under further examination. Although we were unsuccessful with commercial antisera, synthetic epitope-tagged wildtype or mutant Nmf389 cDNA showed localization to the endoplasmic reticulum when expressed heterologously in COS-7 cells. In summary, although the biological function of Tmem151b or role in neurological disease of the highly-conserved human orthologue (Chr 6p 21.1) is unknown, its significant loss of function effect on electroconvulsive threshold and other behaviors in mice suggests TMEM151B as a novel ER protein involved in brain development or function.

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Poster

689. Epilepsy: Genetics and Genetic Models

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Support: NIH Grant GM88804

NIH Grant NS82001

Title: Neuronal hyperexcitability and seizure-like motor pattern generation arising from the Shaker Wings-Down mutation in the *Drosophila* sodium channel gene paralytic

Authors: *A. IYENGAR[1], A. UEDA[1], J. KASUYA[1], S. GRATZ[2], K. O'CONNOR-GILES[2], T. KITAMOTO[1], C.-F. WU[1];
[1]Univ. of Iowa, Iowa City, IA; [2]Univ. of Wisconsin, Madison, WI

Abstract: Mutations in genes encoding voltage-gated sodium (Na_V), potassium (K_V), and calcium (Ca_V) channels are associated with a variety of heritable epilepsy syndromes. In this report, we present a novel hyperexcitable mutant, *Shaker-wings-down* (*Swd*) that displays severe ether-induced shaking and convulsions. We mapped *Swd,* to a single non-polar amino acid substitution in the D3-S4 voltage sensor of *paralytic,* the only gene encoding Nav channels in *Drosophila.* At the larval neuromuscular junction (NMJ), we found extreme excitability indicated by aberrant spontaneous firing in motor axons reminiscent of the K_V double mutant *eag Sh*, but normal synaptic transmission properties. Although spontaneous firing is abolished by TTX in both mutants, electrotonic stimulation of *eag Sh* nerve terminals results in large and sustained neurotransmitter release. In contrast, electrotonically evoked neurotransmitter release in *Swd* was similar to WT in terms of size and time course.
The observed neuronal hyperexcitability in *Swd* led to uncoordinated walking, abnormal motor program outputs, and aberrant spike discharges across the nervous system. Consistent with the observations at the larval NMJ, recordings of adult flight muscle revealed ectopic firing. This spontaneous activity consisted of rhythmic firing (8 Hz) punctuated by brief bursts exceeding 100 Hz. Interestingly, we observed a high degree of bilateral coordination between burst discharges, suggesting a central origin of these seizure-like events.
To demonstrate that the entire functional consequences, from neuronal hyperexcitabilty to seizure behavior, arose from a single amino acid substitution in the Na_V channel mutation, we adopted a CRISPR-based genome editing strategy. The characteristic behavioral and electrophysiological phenotypes of the *Swd* mutation is recapitulated by CRISPR genome editing of the same amino acid replacement. This rules out contributions from unknown genetic background effects and defines a neomorphic mutation of the *paralytic* Na_V channel gene that results in a unique hyperexcitability phenotype. Together, these findings represent a potentially

important mechanism within the Na_V channel that leads to specific neuronal hyperexcitability and neural circuit dysfunction as delineated by the *Swd* mutation.

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Poster

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Topic: B.11. Epilepsy

Support: PAPIIT-DGAPA-UNAM IN215114

Title: Vascular hyperplasia & probable conversion of type I Lafora bodies into type II occurring in the neuropile of Lafora mice

Authors: *J. MACHADO-SALAS[1], M. AVILA-COSTA[2], C. WORBY[3], P. GUEVARA[4], R. M. DURÓN[5], J. ESPINOSA-VILLANUEVA[2], M. TANAKA[6], A. DELGADO-ESCUETA[6]; [1]Ctr. Regional De Daño Cerebral, HAT. Suite 511, 5o. Piso, Torre De Consultorio, TIJUANA, Baja California., Mexico; [2]Neurosci., UNAM Neuromorphology Lab., Mexico, Mexico; [3]Dept. of Pharmacol., UCSD, San Diego, CA; [4]Natl. Inst. of Neurol. and Neurosurg. "Manuel Velasco Suárez", Mexico, Mexico; [5]Facultad de Ciencias de la Salud, Univ. Tecnológica Centroamericana, Tegucigalpa, Honduras; [6]Epilepsy Genetics/Genomics Laboratories, Epilepsy Ctr. of Excellence, Neurol. & Res. Service, West Los Angeles Med. Ctr. and David Geffen Sch. of Med. at UCLA, Los Angeles, CA

Abstract: Here, we define the microscopic features of Lafora Bodies (LfBs), emphasizing the histologic profile and the extra- and intraneuronal distribution of LBs. We provide strong evidence to support a putative tropism between Type I LfBs and blood vessels, which cross the vascular wall and become intraluminal particles, ready to be taken away by the blood flow. Moreover, the presence of vascular hyperplasia in several parts of the brain of the Lafora mice, especially in the ventral pons, suggests that hyperplasia is a response to the high metabolic activity. We also observed thickening and darkening of neurofilamentous elements of the cytoskeleton in the external ring of type II LfBs. This finding establishes a potential relation between type II LfBs and the neurocytoskeleton. Persistence of the circular pattern shown by the type II LfBs allows us to say that these intracytoplasmic inclusions are spherical. Detailed neuropile microscopic scrutiny in which type II LfBs are present lead us to discover a morphological sequence that suggests the transformation of some type I LfBs into type II LfBs.

This process involves a dynamic 3D mechanism that transforms an amorphic, uniformly stained inclusion into a delicate and organized intrasomatic type II LfB. The vascular hyperplasia could be related to an tropism. Finally, how and why do type I LfBs penetrate these vessels and what is their destination? Is this a natural way for getting rid of these inclusions? Is it possible to enhance this mechanism for the clinical improvement of Lafora Disease patients?

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Poster

689. Epilepsy: Genetics and Genetic Models

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Topic: B.11. Epilepsy

Title: Phenotypic and transcriptional differences resulting from two different non erythrocytic spectrin mutations

Authors: *P. R. LEE, JR[1], E. BOSLET[1], V. GARTNER[1], T. MARKELLO[1], A. THURM[2], M. MAEDA[2], G. GOLAS[1], C. TIFFT[1], W. GAHL[1];
[1]NHGRI/Undiagnosed Dis. Program, [2]NIH, Bethesda, MD

Abstract: Previous studies have linked Early Infantile Epileptic Encephalopathy type 5 (EIEE5) with *de novo* mutations in Spectrin Alpha Non-Erythrocytic 1 (SPTAN1). These earlier studies suggest that EIEE5 is a result of mutations that are dominant-negative in nature. The aim of our study was to understand the etiology of disease seen in two additional patients diagnosed via Whole Exome Analysis for *de novo* mutations in SPTAN1 who have severe neurological phenotypes but who do not have seizures. The first of these two cases is a 13-year-old Caucasian male who is heterozygous for a *de novo* nonsense mutation leading to a protein truncated in exon 21. He has global microcephaly, intellectual disability, autism spectrum disorder, and documented thyroid abnormalities, but he has never had an epileptic episode. The second case is a 10-year-old Caucasian male who is heterozygous for a *de novo* intronic missense mutation that results in a premature splice acceptor site and the insertion of five amino acids in the C-terminus of this protein. He has cerebellar and brain stem hypoplasia on MRI, hypothyroidism, intellectual disability, hypotonia, ataxia, autism spectrum disorder, and ADHD, but no epilepsy. The neuroanatomy of patient two is more similar to patients previously described in the literature except he does not have the hypomyelination seen in other patients. The second patient also has a more severe phenotype than the first patient described here, although less severe than those with

EIEE5. Multiplexing digital PCR assays were designed to detect both mutant and wild type alleles, specific for each patient at the site of their mutations. Assays were performed on fibroblast cDNA from cultured patient skin biopsy samples. Results of these assays indicate significant down regulation of wild type and mutant SPTAN1 allele expression in both patient cell lines, suggesting haploinsufficiency may be an alternative etiology, or an additional factor important for understanding disease caused by SPTAN1 mutations.

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Poster

689. Epilepsy: Genetics and Genetic Models

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Topic: B.11. Epilepsy

Title: Pathogenic aspects of SCN1A haploinsufficiency in human ipsc derived cortical neurons.

Authors: ***D. SIMKIN**[1,2], G. L. ROBERTSON[2], E. KISKINIS[2], A. L. GEORGE, Jr.[1];
[1]Pharmacology, Feinberg Sch. of Med., [2]Neurology, Feinberg Sch. of Med., Northwestern Univ., Chicago, IL

Abstract: More than 1100 different heterozygous epilepsy-associated *SCN1A* mutations have been identified, (Claes et al., 2009) with more than 70% occurring in children with Dravet syndrome (DS), a condition characterized by onset of seizures during the first year of life with an ensuing epileptic encephalopathy consisting of cognitive, behavioral and motor impairments, brain malformations as well as premature death (Scheffer et al., 2001, Dravet et al., 2011, Dravet and Oguni, 2013, Brunklaus and Zuberi, 2014). In Dravet syndrome, *SCN1A* loss-of-function is the established molecular mechanism. However, the specific mechanisms explaining how stable genetic mutations in human ion channels cause paroxysmal dysfunction and how the early age of seizure onset affects neurodevelopment remain to be determined. Recent advances in the generation of patient-specific human induced pluripotent stem cells (hiPSCs) have opened the door to elucidating human pathogenic mechanisms of genetic disorders such as the epileptic channelopathies. Hence, we aimed to establish a developmental timeline of pathogenicity associated with *SCN1A* epileptic channelopathies using hiPSC-derived neurons. We have assessed the functional electrophysiological phenotypes of excitatory glutamatergic neurons differentiated from stem cells (Zhang et al., 2013) with haploinsufficient *SCN1A* expression as compared to isogenic controls generated through CRISPR/Cas9 gene editing and healthy controls. We assessed the trajectory of neuronal differentiation and maturation, using current-

clamp electrophysiology and multi-electrode array technology (MEA; Axion Biosystems), at three time-points in differentiation/maturation. Our data suggest that passive properties such as RMP and input resistance mature over time in both DS and control neurons. However, in the first time point of differentiation there was a significant difference between DS and control neurons for both RMP and input resistance. Moreover, active excitability properties such as rheobase and fAHP were enhanced in the DS neurons. Patch-clamp results are consistent with MEA network activity studies showing enhanced excitability in DS neuronal cultures. These data suggest that a haploinsufficiency of *SCN1A* may have cell autonomous effects on the time course of cortical excitatory neuron development leading to enhanced excitability. These important findings help establish an alternative developmental approach to childhood epileptic channelopathies and propel further research into cell autonomous and non-autonomous effects of *SCN1A* haploinsufficiency.

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Poster

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Support: Dravet Syndrome Foundation

SCN2A Research Foundation

Title: Electrophysiological characterization of two Nav1.2 mutations implicated in epilepsy

Authors: *E. MASON[1], R. PATEL[2], Y. XIAO[1], T. R. CUMMINS[1];
[1]Pharmacol. and Toxicology, [2]Stark Neurosciences Res. Inst., Indiana Univ., Indianapolis, IN

Abstract: Mutations in the genes encoding voltage-gated sodium (Nav) channels have been implicated in several types of epilepsy, including many mutations in the alpha subunit of Nav1.2, a sodium channel isoform found primarily in excitatory cortical neurons. These mutations are believed to cause seizures via inducing hyperexcitability and repetitive firing in these neurons. We characterized two such arginine-to-glutamine Nav1.2 mutations, R853Q and R1882Q, in HEK cells. These mutations affect different regions of the channel structure, so we hypothesized that they differentially alter channel function and kinetics. Voltage-clamp studies revealed loss of function resulting from the R853Q mutation as evidenced by lower current density, a faster rate

of inactivation, and a negative shift in the voltage dependence of inactivation compared to wild-type Nav1.2 channels. This channel mutation also enhanced gating pore currents. These currents, generated by ions entering the cell through a gap in the voltage-sensor rather than the central pore, provide a gain of function with this mutation. The R1882Q mutant channel exhibited only gain-of-function effects, including increased current density, enhanced persistent currents, a slower rate of inactivation, and a positive shift in the voltage dependence of inactivation compared to the wild-type Nav1.2. This mutation also increased the amplitude of resurgent currents induced by a Navβ4 peptide in the intracellular patching solution. These observations suggest that the R853Q and R1882Q mutant Nav1.2 channels contribute to the generation of epileptic seizures via different gain-of-function mechanisms, and that treatment of epileptic patients with these mutations may require different pharmacotherapeutic strategies specifically targeting the associated aberrant current activity.

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Poster

690. Epilepsy: Anticonvulsant Therapies

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George Hewitt Foundation for Biomedical Research

MSCA 707530 EpiMiRgen

Title: Dual and Opposing roles of microRNA-124 in epilepsy are mediated via inflammatory and NRSF mediated pathways

Authors: *G. P. BRENNAN[1], D. DEY[2], Y. CHEN[2], K. PATTERSON[2], H. ALICIA[2], C. DUBE[2], T. Z. BARAM[3];
[1]Physiol. and Med. Physics, Royal Col. of Surgeons Ireland, Dublin, Ireland; [3]Pediatrics, Anat. & Neurobio., [2]Univ. of California Irvine, Irvine, CA

Abstract: Insult-insult formation of epileptic networks involves multiple mechanisms. Intervention studies have identified both dysregulated inflammation and aberrant NRSF/REST activity as contributors to epileptogenesis. However, it remains unclear how epilepsy-provoking insults (e.g., prolonged seizures) induce both inflammation and NRSF and whether common mechanisms exist. Using a high throughput rat model of epileptogenesis we examined miR-124

as a candidate dual regulator of NRSF and inflammatory pathways. We induced status epilepticus (SE) using repeated low dose kainic acid which led to reduced miR-124 expression. ChIP analysis revealed that this repression of miR-124 was mediated by SIRT1-and, in turn, miR-124 repression-via C/EBPα upregulated NRSF. We then tested whether augmenting miR-124 after SE would abort epileptogenesis by preventing inflammation and NRSF upregulation. Epilepsy development was monitored using 24h video EEG for 2 months. SE-sustaining animals developed epilepsy, but supplementing miR-124 did not modify epileptogenesis. Examining this result further, we found that synthetic miR-124 not only effectively blocked NRSF upregulation and rescued NRSF target genes, but also augmented microglia activation and inflammatory cytokines. Thus, miR-124 attenuates epileptogenesis via NRSF while promoting epilepsy via inflammation.

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Poster

690. Epilepsy: Anticonvulsant Therapies

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Topic: B.11. Epilepsy

Title: Effect of newly designed mTOR inhibitors on seizure threshold and target activation in normal versus epileptic mice

Authors: C. BRANDT[1], D. FABBRO[2], P. HILLMANN[2], K. ROEMERMANN[1], A. NOACK[1], *W. LOSCHER[3];
[1]Univ. of Vet. Med., Hannover, Germany; [2]PIQUR Therapeut. AG, Basel, Switzerland; [3]Univ. of Vet. Med. Hannover, Hannover, Germany

Abstract: Dysregulation of the mTOR (mammalian target of rapamycin) pathway occurs in different types of epilepsy, thus rendering mTOR an interesting target for antiepileptic and antiepileptogenic strategies. However, results about the potential benefit of rapamycin are controversial, which may be due to poor brain penetration and inhibition of only mTOR-complex 1 (mTORC1). Newer compounds with improved brain uptake were designed to inhibit both mTORC1 and mTORC2 (ATP-competitive mTOR inhibitors) or to additionally block phosphoinositide 3-kinase (PI3K), a regulator upstream of the mTOR complexes (dual PI3K/mTOR inhibitors).
The efficacy of rapamycin and the rapalog everolimus was compared to two new compounds (Cpd-A and Cpd-B). Cpd-A belongs to the group of ATP-competitive mTOR inhibitors while

Cpd-B is a dual PI3K/mTOR inhibitor. For evaluation of the anti-seizure effect of these drugs, the maximal electroshock seizure threshold test (MEST) was performed in nonepileptic and epileptic mice.
The following aims were pursued: (1) Determination of differences in the anti-seizure properties of the drugs; (2) evaluation of the impact of epilepsy on the outcome of drug treatment; (3) investigation of the activation of the mTOR pathway in epileptic vs nonepileptic epileptic mice; (4) comparison of the inhibitory potency of the test compounds on the mTOR pathway.
Female NMRI mice were subdivided into two groups. One group underwent pilocarpine induced status epilepticus in order to induce development of epilepsy and the other group served as nonepileptic control. Different drug dosages and pretreatment times for rapamycin, everolimus, Cpd-A, and Cpd-B were tested in the MEST test. For evaluating mTOR activation, the phosphorylated ribosomal protein S6 was quantified in the hippocampus using Western blot. Difference in mTOR activation was evaluated in nonepileptic versus epileptic mice. For comparison of drug effects on mTOR activation, phosphorylated S6 was analyzed in mice treated with the most efficacious anti-seizure protocol for each compound determined by MEST test.
S6 phosphorylation was increased in epileptic mice compared to naïve mice indicating an activated mTOR pathway. Unexpectedly we found that drug treatment was more efficient in normal compared to epileptic mice in the MEST test. Among all mTOR pathway inhibitors tested, the dual PI3K/mTOR inhibitor Cpd-B exhibited the most pronounced anti-seizure effect. In both normal and epileptic mice, the two new compounds exhibited the strongest inhibition on S6 phosphorylation. This could indicate an advantage in the treatment of epilepsy compared to rapamycin or rapalogs such as everolimus.

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Poster

690. Epilepsy: Anticonvulsant Therapies

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 690.03/K17

Topic: B.11. Epilepsy

Support: USAMRICD Internal Grant

Title: The S940A residue of the potassium-chloride transporter type 2 (KCC2) regulates the onset of soman-induced seizures in mice

Authors: *L. M. MATSON[1], S. M. MILLER-SMITH[1], H. S. MCCARREN[1], D. H. MORROW[2], T. G. DEEB[2], S. J. MOSS[2], J. H. MCDONOUGH[1], C. D. SMITH[3];
[1]Res. Div., USAMRICD, Aberdeen Proving Ground, MD; [2]Tufts Med. Sch., Boston, MA;
[3]USARIEM, Natick, MA

Abstract: Exposure to nerve agents induces numerous symptoms, including benzodiazepine-resistant seizures within 40 minutes of seizure onset. One hypothesis to explain this resistance is that dysfunction of KCC2, a protein that permits GABA's inhibitory tone by maintaining the Cl- gradient, may decrease benzodiazepine efficacy following prolonged seizures. Seizures rapidly decrease KCC2 levels, which may increase intracellular Cl- concentrations and reduce GABA-mediated inhibition. KCC2 function is regulated by phosphorylation at key residues. Importantly, phosphorylation of S940 (pS940) increases KCC2 activity, whereas phosphorylation of T906 (pT906) decreases KCC2 activity. Recently, we showed that soman-induced convulsions are associated with time-dependent changes in KCC2 phosphorylation. In the hippocampus, soman-induced convulsions increased T906 levels at 15 and up to 60 minutes, but did not alter pS940 levels. In the cortex, soman-induced convulsions increased pT906 and pS940 at 15 minutes; however, concentrations of pS940 were equivalent to saline-exposed mice by 60 minutes. Based on these results, how pS940 is involved in soman-induced convulsions is unclear. In Experiment 1, seizure susceptibility in mice lacking the S940A residue (S940A mutant) was increased with shorter seizure latency compared to wild type (WT) controls. In Experiment 2, we determined the median effective dose (ED50) of diazepam for terminating soman-induced seizures in WT mice. Experiment 3 examined diazepam's ability to terminate seizures in S940A mutant and WT mice. Initial evidence suggests that seizure termination latency is higher in S940A mutant compared to WT mice, though additional subjects are required to determine if S940A regulates diazepam efficacy following soman-induced seizures. Our data suggest that KCC2 reactivation may provide a therapeutic strategy for terminating benzodiazepine-resistant seizures.
Disclaimer: The views expressed in this abstract 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 experiment protocol was approved by the Animal Care and Use Committee at 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, as amended. This research was supported by an ILIR awarded by USAMRICD. H.M. and L.S. were supported by appointments to the Postgraduate Research Participation Program at the USAMRICD administered by the Oak Ridge Institute for Science and Education through an interagency agreement between the US DOE and USAMRMC.

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Poster

690. Epilepsy: Anticonvulsant Therapies

Location: Halls B-H

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Program#/Poster#: 690.04/L1

Topic: B.11. Epilepsy

Support: NHMRC Project Grant APP1065882

Title: Conditional upregulation of the KCC2 membrane transporter in a transgenic mouse and the effects on neuronal excitability *In vitro*

Authors: *C. S. GOULTON[1], K. WANG[1], A. KHOSHABA[1], M. WATANABE[2], J. NABEKURA[3], A. J. MOORHOUSE[1];
[1]Sch. of Med. Sci., UNSW Australia, Sydney, Australia; [2]Dept. of Neurophysiol., Hamamatsu Univ. Sch. of Med., Hamamatsu, Japan; [3]Div. of Homeostatic Develop., Natl. Inst. for Physiological Sci., Okazaki, Japan

Abstract: Normal brain function is critically dependent on maintaining a balance between excitation and inhibition. The K^+Cl^--cotransporter (KCC2) plays an important role in regulating intracellular Cl^- concentration and thereby in the efficacy of $GABA_A$-receptor mediated inhibition. This study characterizes a transgenic mouse in which forebrain-restricted expression of KCC2 in pyramidal neurons could be regulated by doxycycline in the diet. Withdrawal of doxycycline resulted in marked upregulation of KCC2, with the mRNA distribution confirmed using *in-situ* hybridization and the time course of protein upregulation measured using western immunoblotting. Upregulation of KCC2 expression was associated with enhanced KCC2 membrane transport function, as judged by an increase in NH_4^+- mediated fluorescence changes measured in hippocampal brain slices loaded with a pH sensitive fluorophore (AM-BCECF). To assess the functional consequences of increased KCC2 on neuronal excitability, field potentials were recorded from hippocampal CA1 in acute slices. There was no effect on the characteristics of orthodromically evoked responses, with similar input-output relationships and paired-pulse ratios being observed ($p>0.05$). There was also no difference in muscimol concentration-response curves, with a similar concentration for half maximal inhibition (IC50) observed in KCC2-upregulated mice (0.85 ± 1.1 μM vs. 1.34 ± 1.1 μM in controls; $p>0.05$). In contrast, KCC2-upregulation was found to reduce hyperexcitability in a range of *in vitro* seizure models when compared to controls. In the tetanus-induced afterdischarge model, the number of afterdischarges was significantly reduced (0.57 ± 0.5 vs. 11.6 ± 5; $p<0.01$). In the zero Mg^{2+} /high K^+ model, spontaneous discharges had a longer latency to onset (12.4 ± 2 vs. 5.7 ± 1 min; $p<0.01$) and were decreased in frequency (0.14 ± 0.03 vs. 0.39 ± 0.05 Hz; $p<0.01$). Our data indicate that this conditional transgenic mouse can be used to produce a rapid upregulation of functional KCC2 protein *in vivo*. While increasing KCC2 has little effect on basal synaptic

transmission, it reduces the susceptibility of neurons to becoming hyperexcitable. Increasing KCC2 may enable greater Cl^- homeostasis and maintain more efficacious GABAergic inhibition, hence providing a novel strategy to enhance neuronal inhibition. The use of this animal model should enable more specific investigations into the neuroprotective potential of targeting KCC2 for the treatment of conditions such as epilepsy.

Disclosures: C.S. Goulton: None. **K. Wang:** None. **A. Khoshaba:** None. **M. Watanabe:** None. **J. Nabekura:** None. **A.J. Moorhouse:** None.

Poster

690. Epilepsy: Anticonvulsant Therapies

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Program#/Poster#: 690.05/L2

Topic: B.11. Epilepsy

Support: NHMRC Project Grant APP1065882

Title: The effect of upregulated KCC2 expression on chemically induced seizures and diazepam therapy *In vivo*

Authors: *D. L. CHEUNG[1], C. S. GOULTON[1], M. WATANABE[2], J. NABEKURA[3], A. J. MOORHOUSE[1];
[1]Sch. of Med. Sci., UNSW Australia, Sydney, Australia; [2]Dept. of Neurophysiol., Hamamatsu Univ. Sch. of Med., Hamamatsu, Japan; [3]Div. of Homeostatic Develop., Natl. Inst. for Physiological Sci., Okazaki, Japan

Abstract: Chloride influx via $GABA_A$ receptor channels is the primary means of hyperpolarizing inhibitory neurotransmission in the brain. It is critically dependent on a low intracellular Cl^- concentration which is maintained by the Cl^- export activity of the K^+-Cl^- cotransporter (KCC2).
During a seizure event, the balance between excitatory and inhibitory activity is disrupted. Given its central role in Cl^- homeostasis we hypothesized that increased KCC2 activity would improve resistance to seizures by enhancing GABAergic inhibition. We tested this using two acute chemical seizure models, systemic pentylenetetrazol (PTZ) and systemic kainic acid (KA), in control and KCC2 upregulated mice. We used a tetracycline conditional expression mouse to over-express KCC2 following withdrawal of doxycycline from the diet. All experiments were approved by the UNSW Animal Care and Ethics Committee (ref. # 12/147B, 15/136A).
In the PTZ challenge, mice were pre-treated with either diazepam (0.6 mg/kg, IP) or vehicle 15 minutes prior a single PTZ injection (85 mg/kg, IP). Behavioural responses were videotaped and

the experiment terminated 30 minutes later or once mice developed maximal tonic-clonic seizures with hind-limb extension. Seizures were subsequently quantified by blinded scoring according to a modified Racine scale. There was no significant effect of KCC2 upregulation on either the latency or severity of seizures, in both the vehicle and diazepam pre-treated groups. In the KA challenge, mice underwent an escalating dose regime receiving two KA injections (5 mg/kg, IP) administered 1 hour apart. Behavioural seizures were terminated after the second hour by a single diazepam injection (5 mg/kg, IP). EEG, from surgically implanted cortical screw electrodes, was recorded throughout the entire procedure. Seizures were initially quantified by integrating the EEG power spectrum density plots for the two KA periods (45 mins each) and post-diazepam period (20 mins). Mice with KCC2 upregulated by withdrawal of doxycycline prior to electrode implantation still displayed clear KA-induced seizures, although the mean power was modestly decreased (49.22 ± 9.223, mean ± SEM, n=10) as compared to control transgenic mice (101.9 ± 39.31, n=10). The efficacy of diazepam in reducing EEG activity in the same cohorts was markedly increased in KCC2 upregulated mice (mean power of 56.78 ± 11.69, n=11), compared to controls (454.2 ± 131.7, n=9).
Our results suggest that increased KCC2 activity by itself has only a limited effect on seizure thresholds but may potentiate the ability of positive GABAergic modulators, such as diazepam, to ameliorate seizures.

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Poster

690. Epilepsy: Anticonvulsant Therapies

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Program#/Poster#: 690.06/L3

Topic: B.11. Epilepsy

Support: (PR) Fondecyt 1130904

Title: Bumetanide enhances the pharmacological effect of phenobarbital on electroencephalographic and behavioral parameters, in an animal model of temporal lobe epilepsy

Authors: *C. A. MANTELLERO[1], C. SALAZAR[2], M. BORQUEZ[2], J. AMARO[2], A. OCAMPO[2], M. INOSTROZA[3], J. VALDES[2], P. ROJAS[1];
[1]Biol., Univ. De Santiago De Chile, Santiago, Chile; [2]Biofísica y Fisiologia, Univ. de Chile, Santiago, Chile; [3]Inst. of medical psychology and behavioral neurobiology, Univ. of tubingen, Tubingen, Germany

Abstract: Temporal lobe epilepsy (TLE) is the most common type of refractory epilepsy to drug treatment. Within the structures involved in TLE, the most important pathophysiologically is the hippocampus, where sclerosis is associated with neuronal loss. This has been related to the cognitive impairment observed in patients. In epileptic conditions neurons have an increased excitability due in part to a reduced synaptic inhibition and a change in the effect of GABA from inhibitory to excitatory. This is due to a change in the direction of electrochemical gradient of Chloride, whose concentration is maintained by the interplay of co- transporters KCC2 and NKCC1. Several studies in experimental models of acute seizures have shown that NKCC1 is highly expressed and KCC2 is decreased, explaining the high intracellular chloride, like in early development. In a model of neonatal hypoxia-induced seizures, Bumetanide, a specific NKCC1 inhibitor, in combination with Phenobarbital shows a significant improvement in seizure control, more effectively than Phenobarbital alone. The aim of this work is to study in an animal model of TLE the refractoriness to a GABAA agonist Phenobarbital, and if in combination therapy with Bumetanide enhances its pharmacological effect decreasing the number of seizures, and a behavioral level reversing cognitive impairment. Male 7 weeks old Sprague Dawley rats were administrated with Pilocarpine to induce Status Epilepticus (SE), and after two weeks they have spontaneous seizures. Animals were administrated with Phenobarbital (N=3), Bumetanide (N=3), or Bumetanide with Phenobarbital (N=3) during two weeks, and electroencephalogram (EEG) recordings were performed before and after this treatment to quantify alterations in epileptiform activity. The results show that Phenobarbital in combination with bumetanide decreases the number of seizures. To determine the effect of these treatments on cognitive impairment, object recognition test were performed, to evaluate episodic memory. Open field was conducted to evaluate anxiety, and last, social interaction was studied to evaluated sign that suggesting depression. The results show that the combination therapy improves behavioral parameters. These findings support the idea that NKCC1 contributes to refractoriness to drug treatment and suggest that Bumetanide might be used as a supplemental therapy in TLE.

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Poster

690. Epilepsy: Anticonvulsant Therapies

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Topic: B.11. Epilepsy

Support: Provost Office, Preminger Scholars Program, and Biology Department at Lafayette College

Title: Characterization of the relationship between diet mitochondrial function and oxidative stress in a fly model of epilepsy

Authors: *E. R. REYNOLDS, A. AUSTIN, K. DELLOVADE, S. NGANGA;
Program in Neurosci., Lafayette Col., Easton, PA

Abstract: *Drosophila* mutants known as "bang-sensitive" have been utilized as models for neurological conditions including epilepsy, sensorineural deafness, and age-dependent neurodegeneration. While the mechanisms producing these phenotypes are unique to each strain, some of the gene products suggest mitochondrial dysfunction and a reduced capacity to tolerate oxidative stress as a possible underlying cause. Diet is an important factor in determining energetics and oxidative stress levels. For example, a ketogenic diet has been shown to be an effective treatment for refractory epilepsy in humans, and antioxidant therapies have been explored in Parkinson's disease. We wanted to more clearly define the connection between diet, mitochondrial function, and reactive oxidative species in this fly model system. Bang-sensitive mutant strains were reared on a standard molasses, yeast and cornmeal (MYC), which is a low protein/high carbohydrate diet, or a protein-rich yeast sugar (1:1 YS) diet. The mutants display a lower percentage of seizures on the YS food, but also reduced viability, lifespan and fecundity. Seizure thresholds were also impacted by the YS diet. Several biochemical methods were utilized to define the effects of diet, including a cytochrome oxidase (CO) assay, a superoxide dismutase (SOD) assay and lipofuscin quantitation. Preliminary results indicate reduced CO activity in mutants as compared to wildtype, with increased CO levels in all flies raised on the YS food. Strain differences were also observed using the SOD assay, however the impact of diet was less clear. However, lipofuscin accumulation appeared to increase in flies raised on YS as compared to MYC. Alterations in mitochondrial function correlate with improvement in epilepsy phenotype, while changes in oxidative capacity may underlie changes in viability.

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Poster

690. Epilepsy: Anticonvulsant Therapies

Location: Halls B-H

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Program#/Poster#: 690.08/L5

Topic: B.11. Epilepsy

Title: Culture medium composition has limited influence on epileptogenesis in organotypic hippocampal cultures

Authors: *J. LIU[1], Y. SAPONJIAN[3,4], M. M. MAHONEY[2], K. J. STALEY[3,4], Y. BERDICHEVSKY[1,2];
[1]ECE, [2]Bioengineering Program, Lehigh Univ., Bethlehem, PA; [3]Dept. of Neurol., Massachusetts Gen. Hosp., Boston, MA; [4]Harvard Med. Sch., Boston, MA

Abstract: Organotypic hippocampal cultures are increasingly used as an in vitro model of post traumatic epilepsy. Chemically defined medium were developed to maintain organotypic cultures. However, it is based on the composition of blood plasma, not of cerebrospinal fluid (CSF). The opening of blood-brain barrier (BBB) due to brain insults may be one of the causes of epileptogenesis in vivo. It is possible that epileptogenesis in organotypic cultures is due to exposure of hippocampal neurons to components of blood serum in the culture medium that are present at different concentration or not present at all in normal CSF. Commercially available culture medium Neurobasal-A (usually supplemented with B27) contains electrolytes, glucose, amino acids and other components that are not present at same concentrations in CSF. We examined the influence of medium composition on epileptogenesis. Slices were maintained in culture media with various compositions. Epileptogenesis was evaluated by measurements of lactate and lactate dehydrogenase (LDH) levels (markers of ictal activity and cell death, respectively) in culture supernatant collected twice a week, immunohistochemistry and automated 3-D cell counts, and extracellular recordings from CA3 and CA1 regions. Changes to culture medium composition moderately reduced lactate and LDH levels as well as electrographic seizure. However, epileptogenesis could not be prevented by composition modification without decreasing the number of surviving neurons. We conclude that medium composition is unlikely to be the cause of epileptogenesis in organotypic hippocampal culture.

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Poster

690. Epilepsy: Anticonvulsant Therapies

Location: Halls B-H

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Program#/Poster#: 690.09/L6

Topic: B.11. Epilepsy

Support: CONACyT 239594

Title: Time-restricted feeding exerts anti-inflammatory and neuroprotective effects on acute seizure model.

Authors: *J. SANTILLAN-CIGALES, J. LANDGRAVE-GOMEZ, O. F. MERCADO-GOMEZ, R. GUEVARA-GUZMAN;
Physiol., Univ. Nacional Autónoma De México, Facultad, Ciudad De Mexico, Mexico

Abstract: Introduction: During the past decade, experimental research has demonstrated a prominent role of the pro-inflammatory molecules on epileptogenic and ictogenic processes. On the other hand, our research group recently demonstrated that time-restricted feeding (TRF) had an anticonvulsant effect, however, the precise mechanism by which this diet exerts its beneficial effects are still unknown. Objective: Our aim was to investigate whether TRF is able to exert its beneficial effect decreasing the expression of pro-inflammatory molecules and thus might have a neuroprotective role after seizure induction. Methodology: Briefly, TRF consisted in allowing rats to eat for two hours daily during their light phase for 20 days; conversely, control group was fed ad libitum (AL). After dietary schedule, status epilepticus (SE) was induced using a pre-treatment with a injection of lithium chloride (3 mEq/kg) followed by pilocarpine administration (60 mg/kg). Both protein and mRNA expression of pro-inflammatory molecules such as interleukin 1 beta (IL-β), tumor necrosis factor alpha (TNF-a) and interleukin 6 (IL-6) were measured in hippocampus from each group 24 h after SE. Additionally, coronal brain slices were processed with fluoro-Jade C to mark degenerate neurons. Results: Our preliminary data showed that the group that followed TRF before SE had a significant decrease in both of mRNA and protein expression of pro-inflammatory molecules (IL-β, TNF-a, IL-6), in comparison with AL group after seizure induction. Furthermore, the hippocampus from TRF group showed a significant decrease on reactive gliosis and less FluroJade-positive cells were observed after SE. Conclusion: Our data demonstrate that TRF may exert a neuroprotective effect by decreasing the mRNA and protein expression of pro-inflammatory molecules and reactive gliosis after seizure induction.

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Poster

690. Epilepsy: Anticonvulsant Therapies

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Topic: B.11. Epilepsy

Support: IPN Grant SIP20151710

Title: Fluorobenzenamides as anticonvulsants

Authors: *S. E. MEZA TOLEDO, J. G. CERVANTES ESPINOZA, M. SUÁREZ QUEZADA, J. PERALTA CRUZ, G. CHAMORRO CEVALLOS;
Dept. de Bioquímica, Escuela Nacional De Ciencias Biológicas, IPN, Ciudad de Mexico, Mexico

Abstract: In an effort to discover novel anticonvulsants, we prepared and characterized several DL-fluorobenzenamides and studied their anticonvulsant activity against seizures induced by 4-aminopyridine, maximal electroshock and pentylenetetrazol in CD-1 mice. Compounds 2-hydroxy-2-(3'-trifluoromethylphenyl)butyramide (**1**), 2-hydroxy-2-(3',5'-bis(trifluoro methylphenyl) butyramide (**2**), 3-hydroxy-3-(3'-trifluoromethylphenyl) pentanamide (**3**) and 2-hydroxy-2-(3',5'-bis(trifluoromethylphenyl) pentanamide (**4**) were prepared using condensation reactions and characterized through infrared spectrophotometry and nuclear magnetic resonance spectroscopy. Each of the four compounds exhibited an anticonvulsant activity in mice greater than that of sodium valproate, a widely-used antiepileptic, against seizures induced by maximal electroshock, 4-aminopyridine or pentylenetetrazol. Compounds **1**, **2**, **3** and **4** were more potent than phenobarbital against seizures induced with 4-aminopyridine and equipotent with phenobarbital against seizures induced by maximal electroshock or pentylenetetrazol. In the rotarod ataxia test, compound **1** exhibited the lowest ataxicity of the four compounds. Incorporation of trifluoromethyl groups in the meta position of the phenyl ring in compounds **1** and **3** increased the duration of their anticonvulsant activity. This study shows that fluorobenzenamides represent a new class of anticonvulsant compounds worthy of further development for potential antiepileptic therapy.

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Poster

690. Epilepsy: Anticonvulsant Therapies

Location: Halls B-H

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Topic: B.11. Epilepsy

Title: Phenytoin halts convulsions, but not electrographic seizure activity, in a rat model of status epilepticus, whereas both are eliminated by next generation neuroactive steroids

Authors: *G. M. BELFORT, A. ALTHAUS, R. S. HAMMOND, M. C. QUIRK, G. MARTINEZ BOTELLA, F. G. SALITURO, A. J. ROBICHAUD, J. J. DOHERTY;
Sage Therapeut., Cambridge, MA

Abstract: Established status epilepticus (SE) is a life threatening condition characterized by unremitting seizures that have failed to respond to first line therapy with benzodiazepines. We report pre-clinical results comparing the electrographic activity of a standard second line anti-epileptic drug with next generation neuroactive steroids and demonstrate the differentiation of these novel potential treatments for SE. Phenytoin is a commonly used second line antiepileptic drug that blocks voltage gated sodium channels. Here we investigated the effect of phenytoin in the rat lithium-pilocarpine model of SE with EEG recording. Phenytoin (5, 15 and 50 mg/kg, IV) administered within 2 minutes of SE onset failed to reduce aberrant EEG spike probability at all doses notwithstanding brain concentrations as high as 37±4.2 μg/g. Despite this lack of effect on EEG, dose dependent sedation and arrest of behavioral convulsions was observed. All animals that received 50 mg/kg exhibited a loss of righting reflex (LRR) up to 30 minutes after dosing and 3 of 4 animals exhibiting LRR for 4 hours. Lamotrigine (3, 10 and 30 mg/kg, IV), another antiepileptic drug that targets sodium channels, also failed to reduce spike probability at all doses when administered immediately after SE onset, despite brain concentrations as high as 26.5±0.6 μg/g. Unlike phenytoin, lamotrigine was not sedating. In contrast to phenytoin and lamotrigine, next generation neuroactive steroid anticonvulsants, reduced aberrant spike probability immediately after SE onset, and retained this activity even when administered up to 60 minutes later. Taken together, these data highlight the discrepancy between behavioral and electrographic measures of ongoing seizure activity and illustrate the potential value of EEG for uncovering ongoing SE in non-convulsive patients. Finally, the sustained activity of next generation neuroactive steroids after SE onset in the lithium-pilocarpine model supports assessing the activity of this class of compounds in humans with established SE.

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Poster

690. Epilepsy: Anticonvulsant Therapies

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Korea Healthcare Technology R&D Project, Ministry of Health & Welfare HI15C1928

Title: Naringenin ameliorates kainic acid-induced morphological alterations in the dentate gyrus in a mouse model of temporal lobe epilepsy

Authors: *J. PARK[1,2], H. JANG[1,2], K. JEONG[1,2], U. JUNG[4], S. KIM[1,2,3];
[1]Sch. of Life Sci. & Biotech., [2]BK21 plus KNU Creative BioResearch Group, [3]Brain Sci. and Engin. Inst., Kyungpook Natl. Univ., Daegu, Korea, Republic of; [4]Dept. of Food Sci. and Nutr., Pukyong Natl. Univ., Busan, Korea, Republic of

Abstract: Granule cell dispersion (GCD) in the dentate gyrus (DG) of the hippocampus is a morphological alteration characteristic of temporal lobe epilepsy. Recently, we reported that treatment with naringin, a flavonoid found in grapefruit and citrus fruits, reduced spontaneous recurrent seizures by inhibiting kainic acid (KA)-induced GCD and neuronal cell death in mouse hippocampus, suggesting that naringin might have beneficial effects of preventing epileptic events in the adult brain. However, it was still unclear whether the beneficial effects of naringin on the inhibition of GCD could be mediated by naringenin, a major metabolite of naringin, in the KA-treated hippocampus. To investigate this possibility, we evaluated whether intraperitoneal injections of naringenin could mimic naringin-induced effects against GCD formation following intrahippocampal KA injections in mouse brains. Our results showed that treatment with naringenin delayed the onset of KA-induced seizures and attenuated KA-induced GCD. Moreover, we found that its treatment inhibited the activation of mammalian target of rapamycin complex 1 (mTORC1) both in neurons and reactive astrocytes in DG of KA-treated hippocampus. These results suggest that naringenin may be an active metabolite of naringin, which has a capacity to control GCD formation, resulting in preventing the progression of epileptic insults in the hippocampus *in vivo*.

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Poster

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Korea Healthcare Technology R&D Project, Ministry of Health & Welfare HI15C1928

Title: Pharmaceutical effects of naringin in the kainic acid-treated animal model of epilepsy

Authors: *H. JANG[1,2], K. JEONG[1,2], M.-T. JEON[1,2], E. LEEM[1,2], U. JUNG[4], S. KIM[1,2,3]; [1]Sch. of Life Sci. & Biotech., [2]BK21 plus KNU Creative BioResearch Group, [3]Brain Sci. and Engin. Inst., Kyungpook Natl. Univ., Daegu, Korea, Republic of; [4]Dept. of Food Sci. and Nutr., Pukyong Natl. Univ., Busan, Korea, Republic of

Abstract: Kainic acid (KA) is well known as a chemical compound to study epileptic seizures and neuronal excitotoxicity, resulting in the death of hippocampal neurons by autophagic stress and microglia-derived neuroinflammation. In addition, epileptic seizures show the abnormal morphological characteristics such as granule cell dispersion (GCD) in the dentate gyrus (DG) of hippocampus, which may be involved in seizure susceptibility and spontaneous recurrent seizure again. Thus, these results suggest that the control of neurotoxic events such as neuroinflammation, excitotoxicity and GCD may be important strategies for inhibition of epileptic events. We recently reported that naringin, a major flavonoid found in grapefruit and citrus fruits, has neuroprotective activities such as anti-neuroinflammation and induction of neurotrophic factors against neurodegeneration in the animal model of Parkinson's disease, suggesting that naringin-induced effects may be useful to other brain diseases. To evaluate the beneficial effects of naringin against epileptic seizures, we have examined the effects of naringin

against KA-induced neurotoxic effects in the hippocampus of mouse brains. Our results showed that intraperitoneal injection of naringin could induce an increase in a threshold for KA-induced seizures, resulting in the delayed onset of seizures, and significantly ameliorated the KA-induced neurotoxicity through anti-autophagic stress and anti-neuroinflammation in the hippocampus compared with KA alone. Moreover, treatment with naringin attenuated the KA-induced mTORC1 activation, which caused GCD in the hippocampus. Therefore, these results suggest that naringin, which induces neuroprotective effects such as anti-autophagic stress, anti-neuroinflammation and anti-GCD, may be a beneficial natural compound against epileptic seizures.

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Poster

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FACES: Finding a Cure for Epilepsy and Seizures

Title: Regulation of excitatory/inhibitory coordination by LPI-GPR55 signaling: implications for seizure generation and management with cannabinoids

Authors: *E. C. ROSENBERG[1], M. BAZELOT[3], A. SALAH[1], B. WHALLEY[3], O. DEVINSKY[2], R. W. TSIEN[1];
[1]Neurosci. and Physiol., [2]Neurol., NYU Sch. of Med., New York, NY; [3]Pharmacol., Univ. of Reading, Reading, United Kingdom

Abstract: Cannabis has been used for centuries as an anti-epileptic therapeutic, and non-psychoactive components of cannabis have reduced seizure activity in both pre-clinical animal models and recent clinical trials (Devinsky et al. 2015). However, the exact mechanism of action of cannabinoids such as cannabidiol (CBD) remains unclear. In one leading hypothesis, CBD acts as a competitive antagonist at the G-protein coupled receptor, GPR55, inhibiting binding of a membrane phospholipid, lysophosphatidylinositol (LPI). CBD blocks LPI-mediated increases in presynaptic Ca^{2+} and consequential vesicular release, thus reducing excitability at excitatory axon terminals (Sylantyev et al. 2013). However, the function of LPI-GPR55 signaling at excitatory postsynaptic sites, inhibitory synapses, and in epilepsy, remains to be explored. Using a multidisciplinary approach, we found that LPI produces distinct pre- and postsynaptic effects at excitatory and inhibitory synapses. At 5-10 min post-application, LPI increased mEPSC frequency in CA1 hippocampus, consistent with previous reports. However, prominent GPR55 immunostaining in CA1 pyramidal neurons cell bodies was also observed, suggesting potential postsynaptic GPR55 expression. LPI elevated GluA1 AMPAR expression at 50-60 min post-application and increased Ser831 and Ser845 phosphorylation. Additionally, LPI reduced inhibitory postsynaptic strength with concomitant decreases in $GABA_AR$ γ_2 subunit expression and Ser327 phosphorylation. Taken together, these results suggest that LPI increases the excitatory/inhibitory ratio in hippocampal neuronal networks by a dual mechanism: enhancing excitatory transmission and attenuating inhibition. This predicts that CBD, by opposing LPI action at GPR55, may exert its beneficial anti-seizure effects at both excitatory and inhibitory synapses.
We further observe that LPI-mediated increases in mEPSC frequency are potentiated in hippocampal slices from animals rendered epileptic following lithium-pilocarpine induced epileptogenesis. Consistent with this finding, acute kainic-acid induced seizures elevated membrane-bound GPR55 expression. Collectively, these results predict a potential positive feedback loop in which seizures elevate GPR55 expression, augmenting the effect of LPI and its seizure-promoting effects on excitatory / inhibitory coordination. Thus, GPR55 represents a potential biomarker for susceptibility to prolonged seizures.

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Poster

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Topic: B.11. Epilepsy

Support: KL2TR001432

Title: Anticonvulsant effect of cannabinoid receptor agonists in a model of neonatal seizures

Authors: *M. HUIZENGA, E. WICKER, V. BECK, P. FORCELLI;
Georgetown Univ., Washington, DC

Abstract: Neonatal seizures and seizures of infancy represent a significant cause of morbidity. 30-40% of infants and children with seizures will fail to achieve seizure remission with current anti-epileptic drug (AED) treatment. Moreover, less than half of electrographic neonatal seizures are effectively controlled with the current first (phenobarbital) or second (phenytoin) line drug treatments. Identification of new therapies for neonatal/infantile epilepsy syndromes is thus of high priority.
Recent case reports have appeared suggesting that cannabis extracts, such as cannabidiol, may exert clinical anticonvulsant effects in young patients. While drugs targeting the cannabinoid system (e.g., CB1 receptor agonists) display anticonvulsant efficacy in adult animal models of seizures/epilepsy, they remain unexplored in neonatal models. However, the cannabinoid system emerges and is active early in development across species, supporting the investigation of targeting this system in neonates. Therefore, we examined the therapeutic potential of drugs targeting the cannabinoid system in a neonatal rodent model of seizures. Postnatal day (P) 10 or P20 male, Sprague-Dawley rat pups were challenged with the chemoconvulsant DMCM (300 ug/kg) 20 min after treatment with either: CB1/2 mixed agonist (WIN 55,212-2), CB1 agonist (ACEA) or antagonist (AM-251), CB2 agonist (HU-308) or antagonist (AM-630), FAAH inhibitor (URB597), and GPR55 agonist (O-1602). Only the mixed CB1/2 agonist and the CB1 agonist displayed anticonvulsant effects in this model. The severity of seizures was significantly decreased and the latency to behavioral seizure symptoms was increased. These effects were seen in P10 animals but not P20 animals. By contrast, both CB1 and CB2 antagonism increase seizure severity.
Together, these results indicate that in neonates, anticonvulsant action of the cannabinoid system is specific to CB1 receptor activation during a specific developmental window. These data provide justification for further examination of CB1 receptor agonists as novel antiepileptic drugs targeting epilepsy in infants and children.

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Poster

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Topic: B.11. Epilepsy

Support: PIFI 231677/2016

Title: Effect of Krill Oil on seizures induced by pentylenetetrazole in adult rats with postnatal seizure induction

Authors: *F. V. VILLALPANDO VARGAS[1], A. M. LARA-VÁZQUEZ[1], L. MEDINA-CEJA[1], L. FLORES-MANCILLA[2];
[1]Biologia Celular y Mol., Univ. of Guadalajara, Guadalajara, Mexico; [2]Univ. Autonoma de Zacatecas, Zacatecas, Mexico

Abstract: Epilepsy is a disease characterized by an enduring predisposition to generate epileptic seizures and by the neurobiological, cognitive, psychological, and social consequences of this condition. It affects 50 million people all over the world. The Krill oil (KO) is an extract of the Antarctic crustacean, *Euphausia superba*, contains phospholipids and omega-3. However, the anticonvulsive effect of the KO has not been reported in literature before. Accordingly, we evaluated its effect on pentylenetetrazole (PTZ) model of generalized seizures, at behavioral level. Male Wistar rats with chronic intragastric administration of Palm oil (300 mg/kg, n=4), water (n=4) and KO (300 mg/kg, n=6), from birth to the end of the experiment, approximately 40-60 postnatal days (PD) and with febrile seizure induction at 4-5PD were used for control and experimental groups. After PTZ administration (90 mg/kg, i.p.) we evaluated the survival period, duration of seizures, loss of posture and convulsive behavior according to Pohl and Mares scale (1987): phase 0.5 sniffing, extensive washing, orientation; phase 1 isolated myoclonic jerk; phase 2 atypical (unilateral or incomplete) clonic seizure; phase 3 fully developed bilateral forelimb clonus; phase 3.5 forelimb clonus with a tonic component and twist of body; phase 4 refers to tonic-clonic seizure with suppressed tonic phase; only clonus of all limbs; phase 5 fully developed tonic-clonic seizure. The results showed a delay in the onset of phase 2 in animals treated with KO compared with palm oil group ($p<0.05$), also an increase in the survival time post-PTZ was observed in the KO group, however it was not statistically significant. In conclusion, the KO did not show anticonvulsant effect at behavioral level in animals with seizures induced by PTZ.

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Poster

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Topic: B.11. Epilepsy

Support: NIH/NINDS grant NS090076

Title: Pregabalin intervention during development is antiepileptogenic in the transgenic epilepsy model of α2δ-1 overexpressing mice

Authors: *W. ZHANG, D. PRINCE;
Neurol. Dept., Stanford Univ. Sch. of Med., Stanford, CA

Abstract: The alpha2delta-1 subunit (α2δ-1) of voltage-gated calcium channels is a receptor for astrocyte-secreted thrombospondins (TSPs) that promote developmental synaptogenesis. Overexpression of α2δ-1 alone in uninjured transgenic mice (α2δ-1 TG mice) results in increased excitatory connectivity and consequent epileptiform seizures. Gabapentinoids, including pregabalin (PGB), are anticonvulsant/antiallodynic drugs that interrupt TSP binding to α2δ-1 and have antiepileptogenic effects in models of cortical injury. However, little is known about the effects and the critical time window of PGB intervention in transgenic epilepsy models like α2δ-1TG mice. To address these questions, we compared the electrophysiological outcomes of PGB treatment administrated at different developmental times in α2δ-1TG mice. We initially treated TG mice with PGB (50 mg/kg ip daily) from P20 to P34 or from P14 to P28 and obtained whole cell recordings of miniature EPSCs (mEPSCs) in layer V pyramidal cells. There were no significant differences in amplitude or frequency of mEPSCs between treated TG mice and saline-treated controls, indicating that existing hyper-connected excitatory circuits were not affected. We obtained epidural EEGs, rather than *in vitro* mEPSC recordings, from a 3rd group of implanted mice treated with PGB from P7 to P28. EEG signals were filtered (1Hz high-pass; 15Hz low-pass) and a Fourier transform power analysis done. EEGs of both untreated and treated TG mice showed epileptiform discharges with peak frequency at 2-4 Hz, 4 and 5 days after the end of treatment. However, the amplitudes of absolute power at 2-4 Hz were decreased to 1/3-1/2 of controls in the PGB-treated group (0.0012-0.0013 vs 0.0006-0.0009 mV^2/Hz). These results suggest that PGB administration from P7 to P28 is effective in attenuating the epileptiform discharges in α2δ-1TG mice. Taken together, our preliminary data show that early PGB intervention can attenuate epileptogenic outcomes in α2δ-1 TG mice. Results raise the possibility of prevention of epileptogenesis in similar genetic models once receptors and appropriate agonists or antagonists are identified.

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Poster

690. Epilepsy: Anticonvulsant Therapies

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Topic: B.11. Epilepsy

Support: MRC Grant hold by Prof. Richardson

Title: Lamotrigine and levetiracetam exert similar modulation of TMS-evoked EEG potentials

Authors: *I. PREMOLI[1], A. BIONDI[1], D. RIVOLTA[2], M. P. RICHARDSON[1];
[1]Dept. of Basic & Clin. Neurosci., King's Col. London, London, United Kingdom; [2]Sch. of Psychology, Univ. of East London (UEL), London, United Kingdom

Abstract: Antiepileptic drug (AED) treatment failures may occur because there is insufficient drug in the brain or because of a lack of relevant therapeutic response. Until now there is no possibility to measure these factors. It has been recently shown that the combination of transcranial magnetic stimulation and electroencephalography (TMS-EEG) can measure the effects of central nervous system acting drugs in healthy volunteers. The EEG response evoked by TMS, named TMS-evoked EEG potentials (TEPs), comprises a series of positive and negative deflections which were specifically modulated by drugs with a well-known mode of action targeting the inhibitory neurotransmission. Hence, we hypothesised that TMS-EEG can depict fingerprints of two widely used AEDs, lamotrigine and levetiracetam, in healthy volunteers. Fifteen healthy subjects participated in a pseudo randomized, placebo-controlled, double-blind, crossover design, using a single oral dose of lamotrigine (300mg), a voltage-gated sodium channel blocker, and levetiracetam (3000mg), which blocks neurotransmitter release by binding to specific vesicles. TEPs were recorded before and 120 min after drug administration and the effects of the drugs on the potentials amplitudes were statistically evaluated. A non-parametric cluster-based permutation analysis of amplitudes at channel-level was run for placebo, lamotrigine and levetiracetam conditions. Both AEDs increased the amplitude of the negative potential at 45ms after stimulation (N45) and suppressed the positive peak at 180ms (P180), whereas placebo did not cause any significant modulations.We demonstrate for the first time AED-induced modulation of TMS-EEG measures. Despite the different mechanism of action that lamotrigine and levetiracetam exert at molecular level, both AEDs are impacting the TMS-EEG response in a similar way. These TMS-EEG fingerprints observed in controls are candidate predictive markers of treatment response in patients on monotherapy with lamotrigine and levetiracetam.

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Poster

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Topic: B.11. Epilepsy

Support: NIH/NINDS R01 NS077908

NIH/NINDS R21 NS072258

Title: Staged anticonvulsant screening for chronic epilepsy

Authors: *Y. SAPONJIAN[1,2], Y. BERDICHEVSKY[3], K.-I. PARK[4], B. ROACH[5], W. POULIOT[5], K. LU[6], W. SWIERCZ[1,2], E. DUDEK[5], K. STALEY[1,2];
[1]Neurol. Dept., Massachusetts Gen. Hosp., Charlestown, MA; [2]Harvard Med. Sch., Boston, MA; [3]Dept. of Electrical and Computer Engin., Lehigh Univ., Bethlehem, PA; [4]Dept. of Neurol., Seoul Natl. Univ. Hosp. Healthcare Syst. Gangham Ctr., Seoul, Korea, Republic of; [5]Dept. of Neurosurg., Univ. of Utah Sch. of Med., Salt Lake City, UT; [6]Boston Univ. Sch. of Med., Boston, MA

Abstract: Significant limitations are associated with studying seizures induced by acute exposure to convulsants in otherwise normal *in vitro* and *in vivo* preparations, which current anticonvulsant screening programs are predominantly based on. One third of epileptic patients do not respond to the numerous anticonvulsants produced as a result of decades of drug screening and development efforts. The slicing preparation of organotypic hippocampal slice cultures parallels traumatic axonal shear injury and subsequently slices develop a dense recurrent synaptic connectivity that results in the development of spontaneous seizures after 1 week *in vitro*. We present a drug discovery program utilizing a tiered compound screening platform based on chronic epilepsy and spontaneous seizures, with compounds advancing from high-throughput *in vitro* models to low-throughput *in vivo* models. The initial stage utilizes the reproducible, accessible and accelerated course of epileptogenesis in the *in vitro* organotypic hippocampal slice culture model of severe post-traumatic epilepsy to conduct a blind screen of an array of compounds and conditions for anticonvulsant, antiepileptic and neuroprotective effects in chronic epilepsy. Lactate production and release of lactate dehydrogenase into spent culture media were used as biomarkers of seizure activity and cell death, respectively, to quantify epileptogenesis. Compounds exhibiting significant reducing effects were retested and subsequently advanced to a second stage comprised of wash-out screens to differentiate anticonvulsant from antiepileptogenic effects, as well as *in vitro* electrophysiological confirmation. The third stage was comprised of double-blind, crossover-controlled, *in vivo* continuous electrographic monitoring of spontaneous seizures in the kainate model of chronic epilepsy. We have screened 140 compounds (most at multiple concentrations) or combinations

of drugs, in over 400 separate drug-concentration experiments alongside control slices from the same animal. We have identified 79 toxic drug-concentrations and over 40 hits. Most current anticonvulsants had modest effects in the initial *in vitro* stage. The cyclooxygenase inhibitor celecoxib had no effect on chemically-induced acute epileptoform activity in normal brain tissue but exhibited robust anticonvulsant activity in all models of chronic epilepsy. This tiered program comprises a promising strategy for the rapid, staged investigation of drug efficacy in post-traumatic epileptogenesis with the ultimate goal of discovering and developing drugs that prevent or modify epileptogenesis and the progression of epilepsy.

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Poster

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Title: Ionic alterations during apoptosis in the developing hippocampus.

Authors: *T. BALENA, Y. SAPONJIAN, K. J. STALEY;
Massachusetts Gen. Hosp., Charlestown, MA

Abstract: New fluorophores and microscopy techniques have made possible more detailed explorations of the process of neuronal death. In the present study we evaluated the death of neurons in a chronically epileptic in vitro preparation in which multiphoton microscopy could be performed over a period of several days. Organotypic hippocampal slice cultures were made from wild-type C57BL/6J mice, and imaged with transgenic fluorophores as well as the Na^+ dye SBFI. Organotypic slice cultures were prepared on P6 and incubated *in vitro* until use, with SBFI added 24 hours prior to imaging. Two-photon imaging was used to excite SBFI at both Na^+-sensitive and -insensitive wavelengths, allowing for the ratiometric determination of the $[Na^+]_i$. Propidium iodide (PI), an indicator of cell death, was used as a costain in most experiments to exclude damaged neurons from the analysis. Immediately post-trauma, neurons had significantly higher $[Na^+]_i$ than has been reported in undamaged neurons. After a brief recovery period, $[Na^+]_i$ again rose to high levels and remained elevated for days. Elevated $[Na^+]_i$ followed decreased

synthesis of virus-induced fluorescent proteins such as TurboRFP, preceded morphological changes such as cell shrinkage and retraction of processes, and coincided with increases in membrane permeability (allowing for the passive influx of dyes and stains such as propidium iodide). The high $[Na^+]_i$ was mitigated by the activity of Na^+/K^+ ATPases, cation/Cl^- cotransporters, and Na^+/Ca^{2+} exchangers in order to support high rates of transmembrane Na^+ flux during epileptogenesis. Inhibition of COX-2 and the protein Bax significantly lowered $[Na^+]_i$, suggesting that an apoptotic pathway leading to the insertion of permeability pores in the cytoplasmic membrane may be responsible for the rise in $[Na^+]_i$ and related changes. Overall, a stereotypical sequence of events preceded neuronal death by at least several days, beginning with quenched emission of fluorescent proteins, dendritic retraction, elevation in $[Na^+]_i$, and terminal cell shrinkage. ATPase activity and secondary ion transport remained robust throughout this process. We are currently testing whether the mitigation of elevated $[Na^+]_i$ reflects a translationally useful neuroprotective effect or a specific effect on $[Na^+]_i$.

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Poster

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MH097909

P30-HD38985

Title: Inhibition of O-GlcNAcase is anticonvulsive and alters hippocampal O-GlcNAcylation levels in the kainic acid model of temporal lobe epilepsy

Authors: *R. G. SANCHEZ, M. C. RICH, F. D. LUBIN;
Neurobio., Univ. Of Alabama At Birmingham, Birmingham, AL

Abstract: Abnormal brain glucose metabolism contributes to the progression of epilepsy disorders. Temporal Lobe Epilepsy (TLE) is a complex disorder that is associated with glucose hypometabolism. Metabolic *O*-linked N-acetylglucosamine (*O*-GlcNAc) signaling that is downstream of the hexosamine biosynthesis pathway (HBP), a metabolic target of glucose metabolism. In the roundworm *Caenorhabditis elegans*, the fruit fly *Drosophila melanogaster*, rodents and humans, the *O*-GlcNAc transferase (OGT) and *O*-GlcNase (OGA) add and remove

O-GlcNAc groups on proteins, respectively. To date, there is virtually nothing known regarding the role of protein *O*-GlcNAcylation in epilepsy. Here, we examined the contribution of *O*-GlcNAcylation to epileptogenesis using kainate-induced experimental TLE model. We found significant decreases in *O*-GlcNAc and OGT levels in the epileptic hippocampus that was more pronounced in hippocampal regions that may be responsible for a loss of cellular glucose homeostasis. Importantly, we observed similar decreases in *O*-GlcNAc and OGT levels in human epileptic hippocampal tissue compared to aged-matched controls (Average age: 39.6yrs). We found that both neurons and astrocytes express OGT and *O*-GlcNAc at baseline, and that *O*-GlcNAcylation levels in these cell-types are differentially regulated in response to potassium chloride or methionine sulfoximine, respectively. To test the contribution of *O*-GlcNAcylation in epilepsy, we treated epileptic animals with the OGA inhibitor Thiamet-G (TMG). We found that TMG treatment restored protein *O*-GlcNAcylation levels in the rat epileptic hippocampus, reduced epileptiform activity and frequency oscillations in our TLE rodent model. Collectively, these findings demonstrate the therapeutic potential of TMG, and a novel role for protein *O*-GlcNAcylation in TLE.

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Poster

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Title: Contrasting effects of perampanel and midazolam on EEG seizure measures in tetramethylenedisulfotetramine-induced status epilepticus

Authors: *D. ZOLKOWSKA[1], D. J. TANCREDI[2], L. OLSEN[1], P. J. LEIN[3], M. A. ROGAWSKI[1];
[1]Dept. of Neurol., Sch. of Med., [2]Dept. of Pediatrics and Ctr. for Healthcare Policy and Res., Univ. of California, Davis, Sacramento, CA; [3]Dept. of Mol. Biosciences, Sch. of Vet. Med., Univ. of California, Davis, Davis, CA

Abstract: Tetramethylenedisulfotetramine (TETS) is a highly lethal neurotoxic rodenticide that acts as a noncompetitive GABA-A receptor. Severe TETS intoxication in humans produces refractory convulsive status epilepticus (SE). We previously reported that perampanel enhances survival of mice in a TETS SE model. Here we assess the impact of perampanel on EEG

measures in the TETS SE model. For comparison, some animals were treated with midazolam, which also reduces mortality in the model. Mice implanted with EEG electrodes were pretreated with a single dose of riluzole (10 mg/kg, IP) and 10 min later received a lethal dose of TETS (0.2 mg/kg, IP). Riluzole does not inhibit TETS-induced SE but does protect against the lethal effects of TETS in mice. Perampanel (3 mg/kg, IP) and midazolam (1.8 mg/kg, IM) were administered 40 min after the first myoclonic twitch. Each 1 min epoch in the EEG recording during the 45 min period after treatment injection was scored for the presence of: isolated spikes/sharp waves (0.08-0.5 Hz), spikes/polyspikes/sharp waves clusters (0.5-2 Hz), and organized seizures. Rates are expressed as total number of rat-minutes with the observed behavior divided by total number of rat-minutes under observation and compared with Poisson regression models for longitudinal data. Perampanel decreased seizures (RR=0.27, 95% CI: 0.14, 0.55) and spike clusters (RR=0.66, 95% CI: 0.43, 1.01) but markedly increased isolated spikes (RR=1.77 95% CI: 1.34, 2.34). In contrast, midazolam did not significantly reduce the rate of seizures (RR=0.68, 95% CI: 0.37, 1.26) but did reduce isolated spikes (RR=0.29, 95% CI: 0.15, 0.55) and spike clusters (RR=0.51, 95% CI: 0.28, 0.94). Peramperal versus midazolam contrasts were statistically significant for organized seizures (RR=0.40, 95% CI: 0.17, 0.95) and for isolated spikes (RR=6.14, 95% CI: 3.30, 11.43) but not for spike clusters (RR=1.28, 95% CI: 0.66, 2.47). Our results demonstrate that perampanel is highly effective at reducing TETS-induced electrographic seizures but interictal-like activity increases. Midazolam, a benzodiazepine which is often used clinically in the treatment of status epilepticus, is less effective in reducing TETS-induced EEG seizures but effectively suppresses epileptiform activity. Our results indicate major difference between AMPA receptor blockade by perampanel and GABA-A receptor positive modulation by midazolam on the electrographic features of TETS-induced SE. The results suggest that perampanel is superior to midazolam in reducing spread of local activity to form organized seizures but discharges overall increase. The long term implications on outcome remain to be determined.

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Poster

690. Epilepsy: Anticonvulsant Therapies

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 690.23/M6

Topic: B.11. Epilepsy

Support: CONACYT Grant CB-2012-177594

Title: Long-term effects of neonatal monosodium glutamate treatment over the expression level of NCX1-3 and their participation in electrographic seizure activity

Authors: *M. HERNANDEZ-OJEDA, M. E. UREÑA-GUERRERO, P. E. GUTIÉRREZ-BARAJAS, A. I. FERIA-VELASCO, C. BEAS-ZARATE;
Dept. de Biologia Celular y Mol., Univ. De Guadalajara-CUCBA, Guadalajara, Jalisco, Mexico

Abstract: A significant neuronal impairment could lead to epileptogenesis in which cellular and molecular modifications persist and bring sudden and recurrent epileptic seizures. Monosodium glutamate (MSG) applied as neonatal treatment produces excitotoxic neuronal damage with several short- and long-term modifications. Increments in the expression level of NCX3 were observed after that treatment as a short-term effect. NCX3 belongs to a genetic family known as SLC8A1-3 that encoding the proteins called NCX1-3. NCX transporters exchange $3Na^{+}$ and $1Ca^{2+}$, and are mainly implicated in Ca^{2+} homeostasis maintenance. Changes in NCXs expression observed after neuronal damage have been associated with epilepsy development. In this work, NCX1-3 expression level was estimated by western-blotting in the hippocampus and entorhinal cortex of adult (60 days) control and MSG-treated animals (4 mg /kg of body weight, subcutaneously applied at postnatal days 1,3,5 and 7). Significant increments in NCX1 and NCX3 were observed in both cerebral regions of MSG-treated animals with respect to controls without significant changes in NCX2. Then, NCX blocking effects were evaluated on electrographic seizures induced by intracerebroventricular administration of 4-aminopyridine (4AP, 3 nmol). KB-R7943 a benzyloxyphenyl derived, which is three fold more efficiently on NCX3 were used as NCX blocker. In control animals, KB-R7943 (62.5 pmol) administered simultaneously to 4AP increased the seizure electrographic activity observed in the hippocampus and entorhinal cortex with respect to convulsive drug administered alone. However, in MSG-treated animals, KB-R7943 delayed the bursts onset and reduced that activity. Results suggest that NXC blocking could control seizures observed after an excitotoxic neuronal damage.

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Poster

690. Epilepsy: Anticonvulsant Therapies

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Cornell University Ithaca-WCMC seed grant

AES seed grant

Daedalus Fund for Innovation

Title: Optogenetic silencing attenuates the activity of acute focal 4-aminopyridine seizures in mouse neocortex

Authors: *M. ZHAO[1,2], E. R. BAIRD-DANIEL[1], R. ALLEVA[1], J.-Y. LIOU[3], H. MA[1,2], T. H. SCHWARTZ[1,2];
[1]Dept Neurolog Surg, [2]Brain and Mind Res. Inst., Weill Cornell Med. of Cornell Univ., New York, NY; [3]Columbia Univ., New York, NY

Abstract: Epilepsy is a neurological disorder that affects roughly 1% of the population worldwide. Although effective treatments with antiepileptic drugs are available, more than 20% of patients have seizures that are refractory to medical therapy and many patients experience adverse effects. Hence, there is a continued need for novel therapies for those patients. Recently, neuromodulations with the optogenetics toolbox have already been shown to be somewhat effective at treating seizures in animal models of epilepsy and may offer a new hope for these refractory patients. However, more studies showed that optogenetics in seizure control must be carefully considered to avoid its potential negative effects. Here, we combined local field potential recordings and optogenetics to test the function of optogenetic silencing in the acute epileptic model. We stereotaxically injected the eNpHR3.0 or Jaws AAV viral vector with CaMKII promoter into neocortex of adult mouse to inhibit cortical pyramidal neurons. Acute focal seizures were induced by 4-aminopyridine (4-AP, 15mM, 0.5 µl) injection. We utilized the local field potential (LFP) to identify ictal discharge and measure epileptic activity. Photostimulation was performed using a TTL-controlled LED light. Histological studies confirmed the expression of eNpHR3.0 or Jaws in excitatory glutamatergic neurons. The total of 101 seizures was recorded from 9 mice. Following 5-8 weeks of eNpHR3.0 and jaws injections, there was no difference in the duration of seizures between the control and the light stimulation. LFP power, however, decreased from 1895.1 ±238.1 mV^2 to 1317.8±107.3 mV^2 in eNpHR 3.0 group ($p<0.05$, n=4 mice, 50 seizures), and from 2148.3 ± 393.8 mV^2 to 1575.5±486.3 mV^2 in the Jaws group ($P<0.05$, n=5 mice, 51 seizures). Taken together, these results demonstrate that optogenetic silencing of principal network can reduce the seizure activity in neocortex. Furthermore, much work is still needed to improve the strategy of optogenetic therapies for epileptic disorders.

Disclosures: M. Zhao: None. **E.R. Baird-Daniel:** None. **R. Alleva:** None. **J. Liou:** None. **H. Ma:** None. **T.H. Schwartz:** None.

Poster

691. Epilepsy: Human Studies II

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Program#/Poster#: 691.01/M8

Topic: B.11. Epilepsy

Support: NIH R01 NS050229

Title: Identification of novel HCN channel phosphosites in human and animal model epilepsy

Authors: F. A. CONCEPCION[1], A. L. KO[2], J. G. OJEMANN[2], *N. P. POOLOS[3];
[1]Neurol., [2]Neurosurg., [3]Univ. of Washington, Seattle, WA

Abstract: Alterations in hyperpolarization-activated, cyclic nucleotide-gated (HCN) channel expression and function have been reported following status epilepticus (SE) in the pilocarpine-induced epileptic rat model (Jung et al., 2010, 2011; Williams et al., 2015). We have shown in these epileptic rats that the altered HCN properties in hippocampal principal neurons (such as changes in surface expression and current (I_h) amplitude) are regulated by phosphorylation. However, the actual phosphorylation sites (phosphosites) on HCN channel proteins that are altered in epilepsy have not been identified. In our current study, our objective was to identify these phosphosites on HCN1 and HCN2 channels through tandem mass spectrometry (MS). We enriched for HCN1 by immunoprecipitating with a monoclonal α-HCN1 antibody in CA1 hippocampal homogenates from male P90-101 epileptic rats (5-6 weeks post SE, n = 7) and age-matched naïve controls (n = 8). Mass spectrometry analyses revealed 6 phosphorylation sites on HCN1 in both our experimental and control samples, located in the cytoplasmic side of the protein at both the N- and C-terminal regions. No statistically significant differences were observed in the frequency of phosphosite identification in epileptic versus naïve samples. For HCN2 channels, we observed 10 phosphorylation sites. Interestingly, we found a statistically significant reduction in phosphorylation at one phosphosite in the C-terminus in epileptic tissue. Finally, we analyzed HCN channel phosphorylation patterns in neocortical tissue from 6 human patients with refractory epilepsy undergoing resection of the epileptogenic zone. For HCN1 channels, we identified 5 phosphosites in human tissue: 3 which were homologous to rat phosphosites, one which was novel, and one with no corresponding homolog on the rat sequence. For HCN2 channels we found 9 human phosphosites, eight of which corresponded to rat homologs, and one which was novel.
In summary, we have identified novel phosphosites on both HCN1 and HCN2 channels, and provide the first evidence of differential phosphorylation of HCN2 channels in an animal model of epilepsy. Also, we show for the first time that phosphorylation of human neocortical HCN channels occurs at predominantly the same phosphosites as in rat hippocampal HCN channels.

Disclosures: **F.A. Concepcion:** None. **A.L. Ko:** None. **J.G. Ojemann:** None. **N.P. Poolos:** None.

Poster

691. Epilepsy: Human Studies II

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Control tissue was obtained from the NICHD Brain and Tissue Bank for Developmental Disorders at the University of Maryland, Baltimore, MD, contract HHSN275200900011C.

Title: Increased brain expression of tau and hyperphosphorylated tau in human therapy-resistant epilepsy

Authors: *L. JACOBS, C. A. COTO, H. JUUL, M. A. DICHTER, F. E. JENSEN, D. M. TALOS;
Neurol., Univ. of Pennsylvania, Sch. of Med., Philadelphia, PA

Abstract: Temporal lobe epilepsy (TLE) is the most common type of partial epilepsy in adulthood and is often the most treatment resistant. In addition, in TLE patients, epilepsy duration and severity positively correlate to impaired cognition. Recent studies in rodent epilepsy models have demonstrated that tau, a microtubule associated protein, may play a key role in enhancing neuronal excitability, and in both human patients and fmouse models, tauopathies are associated with cognitive impairment. We thus hypothesized that in human TLE hippocampal and cortical tissue, both tau and phospho-tau are expressed at higher levels, as compared to controls and that this might be related to both the hyperexcitability and the cognitive impairment seen in these patients.
We evaluated tau and phospho-tau Ser202/Thr205 (At8) expression by Western blot and immunofluorescent double labeling of human hippocampal and cortical samples from subjects with confirmed therapy-resistant TLE who underwent resective epilepsy surgery (n=8, ages 20-56 years). These were compared to hippocampal and temporal lobe cortex samples from neuropathplogically normal autopsy cases (n=5, ages 20-58 years) as controls.
Western blot quantification demonstrated a significant increase of both total tau (700.2% of

control, p<0.0001) and phospho-tau (238.3% of control, p<0.1) in hippocampal tissue homogenates (n=6). Temporal neocortical samples (n=8) also showed increases in total tau (162.9% of control, p<0.1) and phospho-tau (404.7% of control, p<0.01) proteins. Immunohistochemical analysis revealed increased neuronal expression of tau and phospho-tau in both brain regions. The increased expression was also observed in reactive astrocytes and endothelial cells of microvessels.
Together these results indicate a significant tau pathology in human TLE that may contribute to epileptogenesis and possibly cognitive decline. Our data, corroborated with previous pre-clinical studies, further suggest that targeting tau signaling may represent a novel therapeutic strategy for management of refractory TLE and its cognitive consequences.

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Poster

691. Epilepsy: Human Studies II

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Support: ANR-10-LABX-0087 IEC

ANR-10-IDEX-0001-02 PSL

ERC-322721

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Contract no. 14.6008.21.0001, unique ID project RFMEFI60815X0001

RFBR 15-29-01344

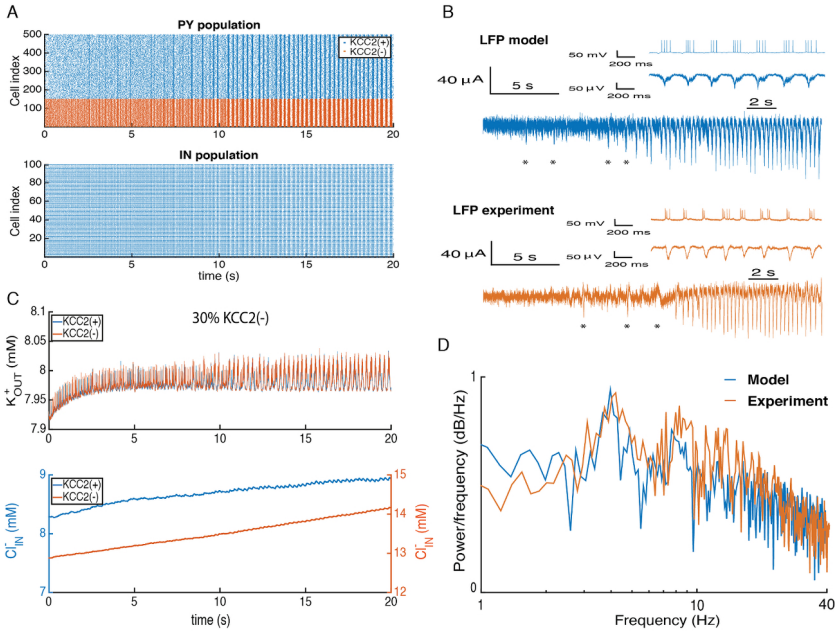
RFBR 15-04-06234

Title: Reduced efficacy of the KCC2 cotransporter promotes epileptic oscillations in a subiculum network model

Authors: *A. BUCHIN[1,3,4], A. CHIZHOV[5,6], G. HUBERFELD[7,8], R. MILES[9], B. GUTKIN[2,4]; [2]Lab. des Neurosciences Cognitives, Group for Neural Theory (France, Paris), [1]Ecole Normale Superieure, Paris, France; [3]Inst. of Physics, Nanotechnology and Communications, Peter the Great St. Petersburg Polytechnic Univ., Saint Petersburg, Russian Federation; [4]Ctr. for Cognition

and Decision Making, NRU Higher Sch. of Econ., Moscow, Russian Federation; [5]Computat. Physics Lab., Ioffe Inst., Saint Petersburg, Russian Federation; [6]Sechenov Inst. of Evolutionary Physiol. and Biochem. of the Russian Acad. of Sci., Saint Petersburg, Russian Federation; [7]Pitié-Salpêtrière Hôpital, AP-HP, Neurophysiol. Dept., Univ. Pierre et Marie Curie, Paris, France; [8]INSERM U1129 "Infantile Epilepsies and Brain Plasticity", Paris Descartes Univ., Paris, France; [9]Cortex et Epilepsie Group, Inst. du Cerveau et de la Moelle Epinière, Paris, France

Abstract: Pharmacoresistant epilepsy is a chronic neurological condition in which a basal brain hyper excitability results in paroxysmal hyper synchronous neuronal discharges. Human temporal lobe epilepsy has been associated with dysfunction or loss of the potassium-chloride co-transporter KCC2 in a subset of pyramidal cells in the subiculum, a key structure generating epileptic activities. KCC2 regulates intra-neuronal chloride and extracellular potassium levels by extruding both ions. Absence of effective KCC2 may alter dynamics of chloride and potassium levels during repeated activation of GABAergic synapses due to interneuron activity. In turn such GABAergic stress may itself affect Cl- regulation. Such changes in ionic homeostasis may switch GABAergic signaling from inhibitory to excitatory in affected pyramidal cells and also increase neuronal excitability. Possibly they contribute to periodic bursting in pyramidal cells, an essential component in the onset of ictal epileptic events. We tested this hypothesis with a computational model of a subicular network with realistic connectivity. The pyramidal cell model explicitly incorporated the cotransporter KCC2 and its effects on the internal/external chloride and potassium levels Our network model suggested the loss of KCC2 in a critical number of pyramidal cells increased external potassium and intracellular chloride concentrations leading to seizure-like field potential oscillations. These oscillations included transient discharges leading to ictal-like field events with frequency spectra as in vitro. Restoration of KCC2 function suppressed seizure activity and thus may present a useful therapeutic option. These simulations therefore suggest that a reduced KCC2 cotransporter activity alone may underlie the generation of ictal discharges.



Disclosures: **A. Buchin:** A. Employment/Salary (full or part-time): École normale supérieure, Paris, Peter the Great St. Petersburg Polytechnic University, NRU Higher School of Economics, Center for Cognition and Decision Making. 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; ANR-10-LABX-0087 IEC, ANR-10-IDEX-0001-02 PSL, FRM FDT20140930942, Contract no. 14.6008.21.0001 unique ID project RFMEFI60815X0001. **A. Chizhov:** A. Employment/Salary (full or part-time): Ioffe Institute, Computational Physics Laboratory. 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; RFBR 15-29-01344,15-04-0623, 16-04-00998. **G. Huberfeld:** A. Employment/Salary (full or part-time): Université Pierre et Marie Curie, Pitié-Salpêtrière Hôpital, AP-HP, Neurophysiology Department, INSERM U1129. 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; ERC-322721. **R. Miles:** A. Employment/Salary (full or part-time): Institut du Cerveau et de la Moelle Epinière, Cortex et Epilepsie Group. 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; ERC-322721. **B. Gutkin:** A. Employment/Salary (full or part-time): École normale supérieure, Laboratoire des Neurosciences Cognitives, Group for Neural Theory, NRU Higher School of Economics, Center for Cognition and Decision Making. 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; ANR-10-LABX-0087 IEC, ANR-10-IDEX-0001-02 PSL, Contract no. 14.6008.21.0001, unique ID project RFMEFI60815X0001.

Poster

691. Epilepsy: Human Studies II

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U.S. Department of Veterans Affairs, Merit Review Award RX000668-01A2

Pablo J. Salame '88 Goldman Sachs endowed Assistant Professorship of Computational Neuroscience

Title: Seizure prediction based on intracortical microelectrode-array LFPs in human focal epilepsy

Authors: *M. AGHAGOLZADEH[1,4], L. HOCHBERG[2,3,4,5], S. CASH[5], W. TRUCCOLO[1,4,2]; [1]Neurosci., [2]Inst. for Brain Sci., [3]Sch. of Engin., Brown Univ., Providence, RI; [4]Ctr. for Neurorestoration and Neurotechnology, U.S. Dept. of Veterans Affairs, Providence, RI; [5]Dept. of Neurol., Massachusetts Gen. Hosp. and Harvard Med. Sch., Boston, MA

Abstract: Novel therapeutic interventions for treating pharmacologically resistant focal epileptic seizures have led to the development of closed-loop systems for seizure control. In a closed-loop seizure control system, electrical stimulation is delivered to prevent seizure initiation or spread once a seizure is detected or predicted to occur imminently. Current systems for seizure prediction/detection have been limited to tracking scalp-based electroencephalogram (EEG) or intracranial EEG (iEEG) signals. Here, we examine seizure prediction based on local field potentials (LFPs) recorded via a 10×10 intracortical microelectrode array implanted in a 4×4 mm^2 neocortical patch in a patient with focal seizures. We formulated the seizure (ictal) prediction problem in terms of discriminating between a period immediately leading to a seizure (preictal) and the interictal neural activity preceding that. In this study, we defined *a priori* the preictal time period as the 5 minute period leading to the ictal onset. We trained convolutional neural networks (CNNs) followed by fully-connected multilayer perceptrons (MLPs) under cross-validation. Input features consisted of the spectral power of LFP channels within consecutive 1-second time windows (0.5-second overlap). We estimated the spectral power within 10 separate frequency bands, namely Δ (0-4 Hz), θ (4-8 Hz), α (8-12 Hz), β_1 (12-18 Hz), β_2 (18-25 Hz), γ_1 (25-50 Hz), γ_2 (50-80 Hz), high frequency oscillations (80-150 Hz), multiunit activity MUA_1 (150-300 Hz), and MUA_2 (300-500 Hz). The dataset included 5 seizures. Seizures were separated by at least 68 minutes. A postictal period of 20 minutes after seizure termination was removed from both the training and testing datasets. The total interictal data spanned 8.12 hours and a leave-one-seizure-out cross-validation approach was used. We show that periods of preictal activity can be successfully discriminated from periods of interictal activity several (2 - 18) minutes prior to seizure onset (80% detection; no false positives). A preictal classification (i.e. seizure prediction) made before the defined preictal period was considered correct if it extended continuously until the seizure onset time. Our preliminary results show that intracortical LFPs may be a promising neural signal for seizure prediction in human focal epilepsy.

Disclosures: **M. Aghagolzadeh:** None. **L. Hochberg:** None. **S. Cash:** None. **W. Truccolo:** None.

Poster

691. Epilepsy: Human Studies II

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Support: NINDS R01NS079533

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U.S. Department of Veterans Affairs, Merit Review Award RX000668-01A2

Pablo J. Salame ’88 Goldman Sachs endowed Assistant Professorship of Computational Neuroscience

Title: A modular platform for closed-loop, low-latency intracranial stimulation in people with neurological disorders

Authors: *A. A. SARMA[1,2,4], B. CROCKER[5], S. S. CASH[6,7,5], W. TRUCCOLO[3,2,4]; [1]Sch. of Engin., [2]Inst. for Brain Sci., [3]Neurosci., Brown Univ., Providence, RI; [4]Ctr. for Neurorestoration and Neurotechnology, Providence VA Med. Ctr., Providence, RI; [5]Hlth. Sci. and Technol., MIT, Boston, MA; [6]Neurol., Massachusetts Gen. Hosp., Boston, MA; [7]Neurol., Harvard Med. Sch., Boston, MA

Abstract: Closed-loop electrical stimulation of the brain can be used to investigate, probe, and potentially treat a range of neurological disorders. The effects of ongoing neural state and dynamics on stimulation response have broad implications for the development of closed-loop neuromodulation approaches. We describe the development of a low-latency platform for pre-clinical, closed-loop neuromodulation studies with human participants. The system can detect neural events in real-time applications on up to 256 electrodes, with a brain-to-stimulation loop latency of 5ms. We illustrate the uses of the platform in exploratory stimulation sessions with individuals with epilepsy undergoing neuromonitoring prior to resective surgery. In these sessions, we tracked interictal epileptiform discharges, estimated as downward crossings of a dynamically computed RMS threshold on the local field potential, to trigger intracranial electrical stimulation. Our preliminary results suggest the relevance of ongoing neural state, at fine timescales, to the stimulation response.

Disclosures: **A.A. Sarma:** None. **B. Crocker:** None. **S.S. Cash:** None. **W. Truccolo:** None.

Poster

691. Epilepsy: Human Studies II

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Topic: B.11. Epilepsy

Support: NIH Grant 2R01NS060757-05A1

Title: Non-invasive low frequency sensory stimulation suppresses seizures in two rodent models of epilepsy

Authors: *N. H. COUTURIER, L. GONZALEZ-REYES, C.-C. CHIANG, D. M. DURAND; Biomed. Engin., Case Western Reserve Univ., Cleveland, OH

Abstract: Rationale: Low Frequency Electrical Stimulation (LFES) has proven to be effective as an alternative treatment for refractory epilepsy. However, LFES requires brain surgery and deep implantation of electrodes in the brain. We investigated whether a non-invasive implementation of this method using low frequency sensory stimulation (LFSS) could provide an effective alternative to surgical resection or electrical stimulation for temporal lobe epilepsy. Methods: The kainic acid animal model of Mesial Temporal Lobe Epilepsy (MTLE) was implemented in order to assess the effect of Low Frequency Audio-Visual stimulation on the frequency and duration of seizures. Stimulation was presented in the form of 1-Hz flashing light and clicking sound. EEG recordings were obtained a week prior to stimulation and compared against EEG recordings during stimulation. Furthermore, evoked potentials were recorded during audio stimulation, visual stimulation, and paired audio-visual stimulation to compare amplitude and latency of each over a 2 hour recording period. Results: A 55% $p<0.001$ (from a baseline of 21.14 ± 1.08 seizures/day to 5.47 ± 0.79 (N=8)) reduction in daily seizures was observed in the kainic acid model. The duration of seizures was also significantly reduced 63% $p=0.0002$. In comparing the evoked potentials from each of the stimulation paradigms all methods demonstrated sustained amplitude and latency over the course of the 2 hour stimulation session. The evoked potential from audio stimulation had an average amplitude of $100\ \mu V \pm 4.58$ and an average latency of $41.1\ ms \pm 0.4$. The paired audio-visual stimulation had an amplitude $142\ \mu V \pm 4.82$ and a latency of $40.3\ ms \pm 0.4$. The visual stimulation had an amplitude of $139\ \mu V \pm 4.11$ and a latency of $60\ ms \pm 1.1$. Conclusion: Significant reduction of seizure frequency/duration indicates that 1-Hz sensory stimulation can decrease excitability and frequency of seizures in the hippocampus. Moreover, the lack of habituation to the stimulation suggests that low frequency sensory stimulation can be effective over long periods of time. Keywords: epilepsy, low frequency stimulation, sensory stimulation, non-invasive treatment. Support: This study was supported by the NIH through grant 2R01NS060757-05A1

Disclosures: **N.H. Couturier:** None. **L. Gonzalez-Reyes:** None. **C. Chiang:** None. **D.M. Durand:** None.

Poster

691. Epilepsy: Human Studies II

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Program#/Poster#: 691.07/M14

Topic: B.11. Epilepsy

Support: CONACyT -PhD Grant 377494

Title: Temporal Lobe Epilepsy & treatments with antiepileptic drugs modify the expression of the Transcription Factor REST/NRSF in patients

Authors: *V. NAVARRETE, SR[1], S. OROZCO-SUAREZ[2], I. A. FERIA-ROMERO[2], M. A. ALONSO-VANEGAS[3], L. ROCHA[4];
[1]Unit of Med. Res. in Neurolog. Dis., Inst. Mexicano Del Seguro Social, Mexico City, Mexico; [2]Unit of Med. Res. in Neurolog. Dis., Inst. Mexicano del Seguro Social, Mexico City, Mexico; [3]Inst. Nacional de Neurología y Neurocirugía "Manuel Velasco Suárez", Mexico City, Mexico; [4]Pharmacobiology, Ctr. de Investigación y de Estudios Avanzados del Inst. Politécnico Nacional (CINVESTAV), Mexico City, Mexico

Abstract: REST (RE1-Silencer Transcription Factor), also known as NRSF (Neuron Restrictive Silencer Factor), acts at chromatin level as a neural transcriptional repressor of genes. Several studies have shown that after induction of seizures in animal models there is an overexpression of REST/NRSF. At present it is unknown if the expression of REST/NRSF is modified in brain tissue of patients with pharmacoresistant epilepsy. The aim of this study was to determine the expression of the gene coding for REST/NRSF Transcription Factor in hippocampus of patients with pharmacoresistant Mesial Temporal Lobe Epilepsy (MTLE), and investigate if clinical variables, like age, gender, treatments, etc., are associated with the results obtained. mRNA was extracted from hippocampal tissue of patients with intractable MTLE (n=28). The extracted mRNA was re-purified by magnetic bead affinity to ensure integrity and cDNA was synthesized. The levels of expression of the gene encoding REST/NRSF, were evaluated using hippocampal tissue from autopsy as controls (n=7) by real-time PCR. Finally we evaluated the correlation with clinical variables. The results show that gene expression of REST/NRSF in hippocampus of patients with MTLE increases 141.63% compared to autopsy tissue ($p<0.05$). No differences in the expression of REST/NRSF was observed in patients treated with valproic acid (VPA) alone or in combination with other antiepileptic drugs (AEDs) compared to tissue autopsy, whereas in those treated with other AEDs is overexpressed 46.53% ($p<0.05$). In addition, in tissue from

autopsies, there is a negative correlation with age ($r=-0.809$, $p<0.05$), the older a lower expression of REST/NRSF. We conclude that in patients with TLE, the gene encoding REST/NRSF is overexpressed, regardless of the clinical variables, but treatment with VPA prevents this overexpression. In neurologically healthy subjects, the expression of REST/NRSF decreases with aging.

Disclosures: V. Navarrete: None. **S. Orozco-Suarez:** None. **I.A. Feria-Romero:** None. **M.A. Alonso-Vanegas:** None. **L. Rocha:** None.

Poster

691. Epilepsy: Human Studies II

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 691.08/M15

Topic: B.11. Epilepsy

Support: NSF Grant 1406556

Title: Characterization of mesial temporal lobe epilepsy network

Authors: *S. KARUNAKARAN[1], G. P. KALAMANGALAM[2], B. AAZHANG[4], N. TANDON[3];
[2]Neurol., [3]Neurosurg., [1]Univ. of Texas Hlth. Sci. Ctr., Houston, TX; [4]Electrical and Computer Engin., Rice Univ., Houston, TX

Abstract: Rationale: The pathological substrate in mesial temporal lobe epilepsy (MTLE) extends beyond the hippocampus to a network of cortical and subcortical structures. Identification of patient-specific epileptogenic networks is essential for modulating these networks to decrease seizure probability and characterize commonly involved structures in a cohort of MTLE patients. Multiple non-invasive methods including fMRI, scalp EEG, PET have been used to characterize epileptogenic networks. These methods do not have consistent activation or precise localization of interictal spikes and ictal onsets and effectively lack the spatio-temporal resolution to characterize epileptogenic networks. An optimal method for delineating these networks would be to use intracranial recordings in the inter-ictal state. As opposed to seizures, that are less frequent, inter-ictal spikes are numerous and occur over multiple components of the MTLE network, allowing us to estimate the flow of information between different brain regions.
Methods: Intracranial recordings of 1 hour duration with frequent inter-ictal discharges were selected from pre-surgical intracranial recordings of 10 medically refractory epilepsy patients. Conditional probability and latency of propagation of inter-ictal spikes were computed using

pairwise estimates of activity in all channels. These were then used to generate a graph theoretical model between all nodes (channels). A logistic regression model was then used to predict surgically resected channels using directed node degree, number of spikes in each channel and their interaction as predictors. The best predictor from the regression was normalized, thresholded and combined across patients to visualize a grouped MTLE network.
Results: Directed node degree was the best predictor to classify the resected epileptogenic zone, determined by conventional clinical methods based on ictal onsets. The grouped network map in these MTLE patients revealed strong connections between hippocampus, amygdala and regions in frontal lobe, limbic system and temporal lobes.
Conclusions: When compared to imprecise non-invasive techniques and interventional, time-consuming stimulation methods, our method utilizes the routinely collected inter-ictal data to generate a map of the MTLE network. These maps may allow us to i) identify targets of a broader network for neuromodulation ii) identify patients with deviations from the "typical" epilepsy network who may not benefit from a resective procedure targeting medial structures iii) compare inter-ictal maps with a similar network obtained during seizure onset.

Disclosures: S. Karunakaran: None. **G.P. Kalamangalam:** None. **B. Aazhang:** None. **N. Tandon:** None.

Poster

691. Epilepsy: Human Studies II

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 691.09/M16

Topic: B.11. Epilepsy

Support: National Institute of Neurological Disorders and Stroke U01NS090407

Title: Increased damage to autonomic brain regions in patients with left temporal lobe seizure onset

Authors: *R. K. HARPER[1], J. A. OGREN[2], K. W. CHOW[3], R. KUMAR[4], D. S. ELIASHIV[5], J. M. STERN[5], S. D. LHATOO[6], R. M. HARPER[2];
[1]Neurobio., Univ. of California Los Angeles Dept. of Neurobio., Los Angeles, CA; [2]Neurobio., [4]Anesthesiol., [5]Neurol., [3]Univ. of California Los Angeles, Los Angeles, CA; [6]Neurol., Case Western Reserve Univ., Cleveland, OH

Abstract: Introduction: Sudden Unexpected Death in Epilepsy (SUDEP), accounts for up to 17% of deaths in individuals with epilepsy, and more than half of deaths in patients with intractable epilepsy. The mechanisms underlying SUDEP remain unclear, but likely involve

cardiac and/or respiratory dysfunction, which are partially mediated by temporal lobe structures. We used MRI-based analyses to determine if patients with left temporal lobe epilepsy (LTLE) exhibit more injury to autonomic-regulatory areas than healthy subjects or patients with other forms of epilepsy.
Methods: Subjects were 10 patients with LTLE(mean age ± SD: 51 ± 16.6 yrs; 6 female) and 10 age/gender-matched healthy controls (49 ± 10.4 yrs; 6 female). Additionally, we examined 11 patients with complex motor seizures (40 ± 14.1 yrs; 8 female) relative to 11 age/gender-matched healthy controls (40 ± 14.8 yrs; 8 female). Subjects were derived from Case Western Reserve University and the University of California at Los Angeles as part of the Center for SUDEP Research. High-resolution T1-weighted images, collected with a 3.0 Tesla MRI scanner, were subjected to entropy-based analysis to detect tissue homogeneity and randomness, with higher values corresponding to greater randomness, and thus, long-term injury. Images were bias-corrected, entropy maps calculated, normalized to a common space, smoothed, and compared between patients and controls using ANCOVA (covariates age, gender; SPM12, uncorrected threshold $p<0.005$). **Results**: LTLE patients exhibited significantly ($P<0.005$) higher levels of tissue entropy over controls throughout the brain in temporal lobe structures and in autonomic-regulatory brain regions, including the insular cortex, hypothalamus, basal forebrain, dorsal and midline thalamus, cingulate cortex, brainstem, and cerebellum (particularly deep autonomic cerebellar nuclei). LTLE patients exhibited clear bilateral hippocampal injury, despite the left hemispheric onset. Only minimal tissue homogeneity differences appeared in patients with complex motor seizures. Patients with complex motor seizures did not exhibit consistent, major long-term injury throughout the brain, likely a consequence of diverse focal origin. Damage was principally confined to small areas of white matter, thalamus, and occipital and cerebellar cortex.
Conclusion: LTLE patients exhibited damage in regions associated with areas of seizure onset. The marked tissue homogeneity differences in autonomic brain regions of LTLE subjects did not appear in all types of epilepsy, and may contribute to elevated SUDEP risk.

Disclosures: **R.K. Harper:** None. **J.A. Ogren:** None. **K.W. Chow:** None. **R. Kumar:** None. **D.S. Eliashiv:** None. **J.M. Stern:** None. **S.D. Lhatoo:** None. **R.M. Harper:** None.

Poster

691. Epilepsy: Human Studies II

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 691.10/M17

Topic: B.11. Epilepsy

Title: A sparse autoregressive model as feature extraction for seizure prediction in temporal lobe epilepsy

Authors: ***P.-N. YU**, C. N. HECK, C. Y. LIU, D. SONG, T. W. BERGER;
USC, Los Angeles, CA

Abstract: Seizure prediction offers patients with epilepsy a better quality of life by allowing them to remove themselves from a dangerous situation. In addition, seizure prediction opens up the possibility to stop the ictal event. Autoregressive (AR) model has been used to extract the features of the interictal state and the preictal state of EEG data. After the extracted features are recognized by a classifier, the classifier can predict a seizure is coming if the following EEG data is recognized as the preictal state. However, determining which EEG channels to train the classifier is a judicious and subjective issue.
This study proposes using sparse logistic regression as the classifier to statistically determine which EEG channels and AR coefficients to be used in seizure prediction. EEG data with 97 channels were processed through the autoregressive model with order of 6 combined with the standard deviation of the residuals, thus ending up with 679 features. Approximately 100 out of the 679 features were statistically chosen by the sparse logistic regression. The 10 cross-validation results show that the overall prediction error from the in-sample data is around 15%. In this study, autoregressive model with order of 6 are currently used. In the future, we will optimize the order of autoregressive model and hopefully decrease the prediction error.

Disclosures: P. Yu: None. **C.N. Heck:** None. **C.Y. Liu:** None. **D. Song:** None. **T.W. Berger:** None.

Poster

691. Epilepsy: Human Studies II

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 691.11/M18

Topic: B.11. Epilepsy

Support: NIH NINDS U01-NS073557

NIH NINDS R01-NS92882

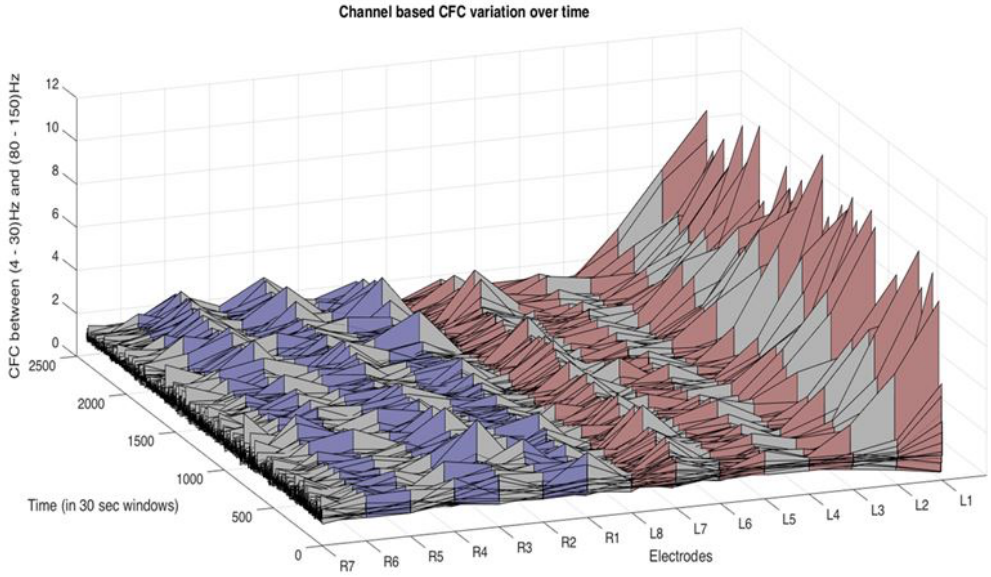
NIH F105355

DARPA-RAM

Title: Interictal phase-amplitude coupling localizes epileptogenic tissue in temporal lobe epilepsy

Authors: *B. M. BERRY[1], M. T. KUCEWICZ[2], J. J. DUQUE[7], Y. VARATHARAJAH[3], B. BRINKMANN[3], V. KREMEN[2], G. C. SIECK[4], S. M. STEAD[2], J. VAN GOMPEL[5], M. R. SPERLING[8], B. C. JOBST[9], R. E. GROSS[10], S. A. SHETH[11], J. M. STEIN[12], D. LEVY[13], S. R. DAS[12], R. GORNIAK[15], D. RIZZUTO[14], M. J. KAHANA[13], G. A. WORRELL[6];
[1]Physiol. & Biomed. Engineering; Neurol., [2]Neurol., [4]Physiol. and Biomed. Engin., [5]Neurosurg., [6]Neurology; Physiol. & Biomed. Engin., [3]Mayo Clin., Rochester, MN; [7]Dept. of Computing and Mathematics, Univ. of Sao Paulo, Sao Paulo, Brazil; [8]Neurol., Jefferson Hosp., Philadelpha, PA; [9]Neurol., Dartmouth Col., Hanover, NH; [10]Neurosurg., Emory Univ., Atlanta, GA; [11]Neurosurg., Columbia Univ., New York, NY; [12]Radiology, [13]Psychology, [14]Sch. of Arts and Sci., Univ. of Pennsylvania, Philadelphia, PA; [15]Radiology, Jefferson Univ., Philadelphia, PA

Abstract: Various interictal electrophysiologic biomarkers now exist as consistent indicators of epileptic brain regions such as High Frequency Oscillations HFOs and Interictal Epileptiform Discharges (IEDs). Phase-amplitude coupling (PAC) to date has not been heavily investigated as an interictal biomarker of seizure onset zone (SOZ). In a series of patients with drug-resistant epilepsy undergoing evaluation for surgery with intracranial EEG monitoring the effect of behavioral state (wake/sleep) and brain region (epileptic/non-epileptic) on PAC was investigated. The gold standard SOZ channels were determined by clinical review and behavioral state determined using simultaneous scalp EEG recordings. We show that spontaneous PAC occurs during interictal time far removed from seizures, and is markedly increased during IEDs. Furthermore, both the variance and the absolute value of PAC within a patient can be used to localize SOZ. The particular sleep state of the patient also appears to correlate with periods of significant changes in both PAC values and within channel PAC variance. Receiver-Operator Curves were used to evaluate the efficacy of localization relative to gold standard and the range of Area-Under-the-Curve (AUC) in this series was 0.52 - 0.90. The spectral characteristics of these PAC were also evaluated and while there was no significant difference seen between low-gamma (30-55Hz), high-gamma (65-100 Hz), or epsilon-gamma (100-150Hz) frequency for amplitude ranges, there was in the alpha and beta frequency for phase ranges. Increases in PAC highly correlate with IEDs. However, after removing the segments containing IEDs localization was still possible. These findings reveal that PAC is increased in epileptic brain, and in many cases can be used as an additional interictal biomarker of SOZ, giving complementary information to currently utilized electrophysiologic measures.



Disclosures: **B.M. Berry:** None. **M.T. Kucewicz:** None. **J.J. Duque:** None. **Y. Varatharajah:** None. **B. Brinkmann:** None. **V. Kremen:** None. **G.C. Sieck:** None. **S.M. Stead:** None. **J. Van Gompel:** None. **M.R. Sperling:** None. **B.C. Jobst:** None. **R.E. Gross:** None. **S.A. Sheth:** None. **J.M. Stein:** None. **D. Levy:** None. **S.R. Das:** None. **R. Gorniak:** None. **D. Rizzuto:** None. **M.J. Kahana:** None. **G.A. Worrell:** None.

Poster

691. Epilepsy: Human Studies II

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Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 691.12/N1

Topic: B.11. Epilepsy

Support: NIH Grant 5R44NS062477-08

Title: Quantification of seizurogenic activity with multiwell microelectrode array technology for proconvulsant risk assessment and disease-in-a-dish epilepsy models

Authors: ***D. C. MILLARD**, H. B. HAYES, A. M. NICOLINI, C. A. ARROWOOD, J. D. ROSS;
Axion Biosystems, Atlanta, GA

Abstract: The lack of advancement in anti-epileptic drugs (AEDs) over the last 30 years, along with the continued need for improved proconvulsant screening in drug safety, motivates the need for new assays of seizurogenic neural activity. Previous work has established an in vitro approach for detecting and quantifying seizurogenic activity using multiwell microelectrode array (MEA) technology, providing a predictive and high-throughput avenue for the evaluation of the efficacy of AEDs and the proconvulsant risk of other drug candidates. Here, we present an updated assay of seizurogenic activity using the Axion BioSystems Maestro multi-well MEA system. We used previously published metrics for the detection of burst spiking events and the quantification of synchronization across a neural population, in spontaneous and evoked conditions. In addition, optogenetic approaches were used to systematically vary the network activity states. Data are included from rat cryopreserved cortical neurons evaluated with 16 compounds, including AEDs (i.e. Carbamazepine, Phenytoin, Tiagabine), reference compounds with known proconvulsant risk (i.e. 4-Aminopyradine, Strychnine, Pentylenetetrazole, Picrotoxin), and negative control compounds. Our results support the combined use of evoked and spontaneous neural activity, collected using multi-well MEA technology, for the high throughput evaluation of complex neuronal networks in vitro to inform the development of AEDs, while also quantifying the proconvulsant risk of candidate pharmaceuticals in a pre-clinical setting.

Disclosures: **D.C. Millard:** A. Employment/Salary (full or part-time): Axion Biosystems. **H.B. Hayes:** A. Employment/Salary (full or part-time): Axion Biosystems. **A.M. Nicolini:** A. Employment/Salary (full or part-time): Axion Biosystems. **C.A. Arrowood:** A. Employment/Salary (full or part-time): Axion Biosystems. **J.D. Ross:** A. Employment/Salary (full or part-time): Axion Biosystems.

Poster

691. Epilepsy: Human Studies II

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 691.13/N2

Topic: B.11. Epilepsy

Support: National Institute of Neurological Disorders and Stroke U01NS090407

Title: Reduced entropy in patients with generalized tonic-clonic seizures

Authors: ***J. A. OGREN**[1], R. KUMAR[2], J. M. STERN[3], D. S. ELIASHIV[3], I. KESELMAN[3], J. ENGEL, Jr.[3], B. DIEHL[4], B. DIEHL[4], S. D. LHATOO[5], R. M. HARPER[1];
[1]Dept. of Neurobio., [2]Dept. of Anesthesiol., [3]Dept. of Neurol., UCLA, Los Angeles, CA;

[4]Neurol., Univ. Col. London, London, United Kingdom; [5]Neurol., Case Western Reserve Univ., Cleveland, OH

Abstract: Rationale: Patients with epilepsy are at risk for Sudden Unexpected Death in Epilepsy (SUDEP); those who exhibit generalized tonic-clonic seizures (GTCs) are at even greater risk. Of recorded SUDEP deaths, a substantial proportion occurred nocturnally after a GTC. Using MRI-based entropy procedures, we assessed tissue texture, indicative of brain changes, in GTC patients relative to healthy controls to evaluate ongoing detrimental processes which may contribute to autonomic or breathing dysfunctions underlying SUDEP. **Methods:** As part of the Center for SUDEP Research, high resolution T1-weighted images were collected with a 3.0-Tesla MRI scanner from 53 patients with GTCs and 53 age- and gender-matched healthy controls at Case Western Reserve University, University College, London, and the University of California at Los Angeles. Images were bias-corrected, entropy maps calculated, normalized to a common space, smoothed, and compared between GTC patients and controls using ANCOVA (covariates age, gender; SPM12, family-wise error correction for multiple comparison, $p<0.01$). **Results:** Decreased entropy values, likely reflecting neuronal swelling, appeared in primary cortical motor/sensory areas, as well as key autonomic sites. The ventromedial prefrontal cortex, hippocampus and surrounding temporal cortex, and the insula also showed lower entropy values. In addition, decreased entropy appeared in the basal ganglia, anterior-medial cerebellum (including the deep autonomic nuclei), and external surfaces of the pons, with the more caudal medulla prominently affected. The anterior and posterior thalamus and midbrain also showed decreases. Only a few isolated regions showed increased entropy. **Conclusions:** Decreased entropy (increased tissue homogeneity, potentially resulting from inflammation or another cause of neuronal swelling) appears in major autonomic regulatory areas (ventromedial prefrontal cortex, insula, hippocampus, dorsal and ventral medulla, deep autonomic cerebellar nuclei) and multiple motor regulatory areas (sensory and motor cortex, basal ganglia, cerebellum), suggesting that GTC seizures have the potential to initiate injury both to autonomic regulatory and motor control sites. Since seizures in GTC patients arise from widespread brain areas, these analyses are unlikely to uncover areas of long-term injury that are consistent across patients, particularly as some have only infrequent GTCs. Future analyses, grouping patients by GTC severity or frequency, may reveal processes contributing to increased entropy, such as which appears in temporal lobe epilepsy.

Disclosures: J.A. Ogren: None. **R. Kumar:** None. **J.M. Stern:** None. **D.S. Eliashiv:** None. **I. Keselman:** None. **J. Engel:** None. **B. Diehl:** None. **B. Diehl:** None. **S.D. Lhatoo:** None. **R.M. Harper:** None.

Poster

691. Epilepsy: Human Studies II

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 691.14/N3

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R01NS070899

NSF CAREER MCB-1252345

Title: A personalized, molecular diagnosis of Lafora epilepsy patient mutations

Authors: *M. S. GENTRY;
Biochem., Univ. of Kentucky, Lexington, KY

Abstract: Of all the severe and intractable epilepsies, Lafora disease (LD) is among the most severe, and is inevitably fatal. Mutations in two genes have been identified that cause LD, *EPM2A* (laforin) and *EPM2B* (malin). *Identification of the genetic basis for LD has opened up a new era in our understanding of the cause of LD, leading to rapid progress in the field.* Mutations in either of these genes cause glycogen to transform into malformed (starch-like), aggregated inclusions called Lafora bodies. These inclusions overtake the cytoplasm of dendrites, and drive the progressive refractory seizure disorder.

The human *EPM2A* gene encodes the phosphatase laforin and recessive mutations in *EPM2A* result in Lafora disease (LD). We previously defined that the physiological function of laforin is to dephosphorylate glycogen. In the absence of laforin activity, glycogen transforms into hyper-phosphorylated, water-insoluble, starch-like inclusions that drive neuronal apoptosis, neurodegeneration, and eventual death of LD patients. LD patient missense mutations are dispersed throughout laforin, bringing to question the structural mechanism(s) of disease.

We recently determined the crystal structure of human laforin at 2.4 Å bound to oligosaccharides with a phospho-glucan product at the active site. The structure reveals an integrated tertiary structure of the carbohydrate binding module and dual specificity phosphatase domains as well as an antiparallel dimer mediated by the phosphatase domain that results in a tetramodular architecture, positioning the two active sites ~31 Å from each other. We utilized the crystal structure and three solution-based, biophysical techniques along with biochemical analyses of LD patient mutations and structured guided mutations to probe this unique tertiary and quaternary structure. We define a cooperative mechanism of action for laforin as well as establish the effect of LD disease mutations, thereby providing atomic level insights that connect basic glycogen metabolism to human neurodegenerative disease.

Cumulatively, this work allows us to provide a patient specific, molecular diagnosis of each LD laforin mutation. This personalized, molecular diagnosis will prove invaluable as our groups and others move towards a LD therapeutic and/or cure.

Disclosures: M.S. Gentry: None.

Poster

692. Demyelinating Disorders: Therapeutics

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 692.01/N4

Topic: B.13. Demyelinating Disorders

Support: Sherman Family Foundation

ERA-Net for Research on Rare Diseases, grant 3000010861

Jerome Lejeune Foundation, grant 203430

German-Israli Foundation, grant 1314

Title: Genetic and pharmacological treatment of hypomyelination in the zebrafish model for psychomotor retardation

Authors: *L. APPELBAUM, D. ZADA, A. TOVIN;
The Fac. of Life Sci. and The Multidisciplinary Brain Res. Ctr., Bar Ilan Univ., Ramat Gan, Israel

Abstract: Hypomyelination is a key symptom of the psychomotor retardation Allan-Herndon-Dudley syndrome (AHDS), which is associated with mutations in the thyroid hormone transporter *mct8*. AHDS is characterized by severe intellectual deficiency, neuromuscular impairment, and hypothyroidism in the brain. In order to study the neurological mechanism and find potential treatments, we developed an *mct8* mutant zebrafish (*mct8*-/-) as a model for AHDS. The transparent zebrafish is a vertebrate with simple yet conserved brain structure and function, and is amenable for live imaging of single cells and molecules. The *mct8*-/- larvae showed neurological phenotypes including pronounced hypomyelination. We found that the expression of genetic markers for oligodendrocyte progenitor cells increased while the expression of markers for mature oligodendrocytes decreased in *mct8*-/- larvae and adults. Two-photon live imaging showed that the number of oligodendrocytes in the brain and the spinal cord is reduced in *mbp:EGFP*/*mct8*-/- larvae and juveniles. Pharmacological treatment with thyroid hormone analogs and other myelin promoting compounds partially recovered myelination in *mct8*-/- larvae. Intriguingly, treatment with the thyroid hormone T3 did not affect myelination in relatively late developmental stages, after the maturation of the blood-brain barrier (BBB), suggesting that T3 can not cross the BBB in the absence of the MCT8 transporter. Thus, we transiently expressed a MCT8-tagRFP fusion protein in the vascular system. This relatively low

mosaic expression of MCT8-tagRFP specifically in endothelial cells of the vascular system completely recovered the number of oligodendrocytes in *mct8*-/- larvae. These results suggest that TH analogs and BBB-targeted gene-therapy can enhance myelination in AHDS and possibly in other thyroid-dependent brain disorders.

Disclosures: **L. Appelbaum:** None. **D. Zada:** None. **A. Tovin:** None.

Poster

692. Demyelinating Disorders: Therapeutics

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 692.02/N5

Topic: B.13. Demyelinating Disorders

Support: Brian's Hope

Title: Nanoparticle therapies for the treatment of X-linked adrenoleukodystrophy

Authors: ***C. L. NEMETH**[1], B. R. TURK[1], O. GOK[2], S. P. KAMBHAMPATI[2], J. S. MARX[1], B. E. THEISEN[1], R. R. RAMIREDDY[2], F. ZHANG[2], M. V. JOHNSTON[1], R. M. KANNAN[2], S. KANNAN[3], A. FATEMI[1];
[1]Dept of Neurol., Kennedy Krieger Inst., Baltimore, MD; [2]Ctr. for Nanomedicine, [3]Anesthesiol. and Critical Care Med., Johns Hopkins Univ., Baltimore, MD

Abstract: X-linked adrenoleukodystrophy (X-ALD) is a devastating, rapidly progressing, demyelinating condition affecting boys with an incidence of about 1:17,000. Symptoms of X-ALD first appear between the ages of 4 and 10 years of age and is almost always fatal within 2-3 years. Hallmark pathophysiology includes accumulation of very long chain fatty acids (VLCFA), increased oxidative stress, and progressive axonopathy. Although a monogenic disease, arising from defects in the peroxisomal membrane transporter protein, ABCD1, it exhibits little to no genotype-phenotype correlation and this phenotypic variability complicates prognosis and treatment. Hematopoietic cell transplant during early-stage disease progression has shown some success, however adjuvant treatments are necessary and otherwise promising pharmacotherapies demand chronic treatments at dangerous doses. Recent advancements in dendrimer nanoparticle therapeutics provide platforms in which dendrimer-drug conjugates enable targeted and intracellular slow release of drugs requiring fewer treatments at lower drug concentrations. Previous work has shown the use of dendrimer-drug conjugates to be a non-toxic and effective mechanism of drug delivery. Moreover, we have demonstrated uptake of dendrimer-drug conjugates by affected cell types in both *in vivo* and *in vitro* models of X-ALD, and the adult slowly progressing form adrenomyeloneuropathy (AMN). Dendrimer conjugates have been

detected within spinal cord neurons of the ABCD1 knockout mouse as well as within patient-derived primary macrophages and fibroblasts. Furthermore, use of the anti-inflammatory/anti-oxidant N-acetyl-L-cysteine (NAC)-dendrimer conjugate (D-NAC) has resulted in reduced levels of inflammation within X-ALD patient macrophages as compared to drug treatment alone. Together, these models recapitulate the hallmark pathophysiology of ALD-related diseases and these findings demonstrate reductions in disease burden following nanoparticle treatment. Nanotherapeutic treatment strategies offer a novel method to effectively and selectively target affected cells and may provide new therapeutic opportunities for complex diseases such as X-ALD.

Disclosures: **C.L. Nemeth:** None. **B.R. Turk:** None. **O. Gok:** None. **S.P. Kambhampati:** None. **J.S. Marx:** None. **B.E. Theisen:** None. **R.R. Ramireddy:** None. **F. Zhang:** None. **M.V. Johnston:** None. **R.M. Kannan:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ashvattha Therapeutics LLC. **S. Kannan:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ashvattha Therapeutics LLC. **A. Fatemi:** None.

Poster

692. Demyelinating Disorders: Therapeutics

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 692.03/N6

Topic: B.13. Demyelinating Disorders

Title: Activation of oligodendroglial PGC-1a by physical exercise accelerates remyelination

Authors: *S. K. JENSEN, N. J. MICHAELS, M. B. KEOUGH, J. N. HAHN, V. YONG; Univ. of Calgary, Calgary, AB, Canada

Abstract: Treatment modalities that promote the functional regeneration of myelin, termed remyelination, remain in their infancy despite considerable investigation. In multiple sclerosis, physical exercise and participation in activities of daily living are associated with reduced disability with little mechanistic rationale. Considering environment-derived stimuli and activity are known to stimulate myelination in the healthy central nervous system, we investigated the capability of physical exercise to promote remyelination during pathology. We show that immediate therapeutic access to a running wheel enhances oligodendrocyte generation following a lysolecithin-induced demyelinating insult. We observe a 39% and 30% increase in oligodendrocytes at 7 and 14 days post lesion (dpl), respectively. At 7 dpl, 50% of all oligodendrocytes are PDGFRα^+ progenitors and label for the proliferation marker Ki67. At 14

dpl, these newly formed progenitors functionally differentiate into $CC1^+$ mature oligodendrocytes, with progenitors making up <20% of all oligodendrocytes. Moreover, exercise increases the capacity for individual oligodendrocytes to form myelin segements resulting in a 2.7 fold increase in the number of myelinated axons at 14 dpl and accelerates the thickening of myelin. We identify PGC-1α as an exercise-induced factor in oligodendrocyte progenitors, mature oligodendrocytes, and select astrocytes. Further, RNA sequencing of micro-dissected lesions reveals increases in key metabolic genes as a result of exercise. We suggest this is a PGC-1α-driven effect, as it is known to coordinate the transcription of metabolic genes in other contexts, and that this increased metabolic capacity in oligodendrocytes enhances remyelination. We are currently investigating this link further. Overall, this study demonstrates that physical exercise is an efficacious means to enhance remyelination and describes a novel PGC-1α-dependant mechanism through which oligodendrocytes respond to physical activity and myelination is coupled to metabolism.

Disclosures: S.K. Jensen: None. **N.J. Michaels:** None. **M.B. Keough:** None. **J.N. Hahn:** None. **V. Yong:** None.

Poster

692. Demyelinating Disorders: Therapeutics

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 692.04/N7

Topic: B.13. Demyelinating Disorders

Support: CIHR

Research Manitoba

Rick Hansen Institute

Title: Neuregulin-1 promotes oligodendrocytes replacement and fosters a pro-regenerative inflammatory response in focal demyelinating lesions of the spinal cord

Authors: *H. KATARIA, A. ALIZADEH, G. M. SHAHRIARY, K. T. SANTHOSH, S. KARIMI-ABDOLREZAEE;
Dept. of Physiol. & Pathophysiology, Univ. of Manitoba, Winnipeg, MB, Canada

Abstract: Oligodendroglial cell death and demyelination are hallmarks of CNS injuries and multiple sclerosis resulting in axonal damage and functional impairments. Restoration of myelin sheath (remyelination) remains a major obstacle as the ability of endogenous neural precursor cells (NPCs) for oligodendrocyte replacement is hindered in the unfavorable milieu of

demyelinating conditions. Our recent evidence in spinal cord injury indicates that drastic downregulation of Neuregulin-1 (Nrg-1), a critical growth factor for development and function of oligodendrocytes, may challenge the endogenous replacement of oligodendrocytes following injury. Here, in a rat model of lysolecithin (LPC)-induced demyelination, we demonstrate that Nrg-1 is also dysregulated in focal demyelinating lesions. We delivered recombinant human Nrg-1β1 (rhNrg-1β1) intraspinally at the time of LPC injections using poly lactic-co-glycolic acid (PLGA) nanocarriers. Spinal cord tissues were analyzed with ELISA, Western blotting and immunohistology at different time-points after LPC injections. Our findings show that rhNrg-1β1 treatment enhances oligodendrocyte and axonal preservation and promotes oligodendrocyte generation and myelin basic protein expression. Interestingly, Nrg-1 also fosters a neuroprotective and pro-regenerative inflammatory response characterized by induced interleukin-10 (IL-10) and arginase-1 (Arg1) expression in LPC-demyelinating lesion. Our complementary *in vitro* studies using brain derived primary rat adult NPCs and adult rat dorsal root ganglion neurons (DRGs) also provided direct evidence that availability of Nrg-1 significantly increases the number of myelin basic protein expressing cells in DRG-NPC co-cultures cells. Mechanistically, our co-immunoprecipitation studies for ErbB receptors in NPCs revealed that Nrg-1 specifically mediates it effects through ErbB4 receptor activation. In conclusion, our work provides new evidence suggesting an impact for Nrg-1 in promoting oligodendrocyte replacement and axonal remyelination in demyelinating lesions of the CNS that is in part attributed to the anti-inflammatory effects of Nrg-1. *Supported by the CIHR, Research Manitoba and Rick Hansen Institute.*

Disclosures: **H. Kataria:** None. **A. Alizadeh:** None. **G.M. Shahriary:** None. **K.T. Santhosh:** None. **S. Karimi-Abdolrezaee:** None.

Poster

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Title: Antisense oligonucleotides (ASOs) targeting mouse GFAP reverse pathology in a model of Alexander disease

Authors: *B. POWERS[1], T. HAGEMANN[2], S. WHEELER[2], C. MAZUR[1], E. SWAYZE[1], A. MESSING[2];
[1]Neurosci. Drug Discovery, Ionis Pharmaceuticals, Carlsbad, CA; [2]Waisman Ctr. and Dept. of Comparative Biosci., Univ. of Wisconsin-Madison, Madison, WI

Abstract: Alexander disease (AxD) is a rare, generally fatal leukodystrophy caused by autosomal dominant missense mutations in the gene for glial fibrillary acidic protein (GFAP). Considerable evidence supports the hypothesis that these mutations act in gain-of-function fashion, with accumulation of GFAP above a toxic threshold causing a cascade of effects both within astrocytes and on other cells of the CNS. GFAP and other proteins accumulate into Rosenthal fibers, the aggregates within cell bodies and processes of astrocytes that are the pathological hallmark of the disease. Seizures, megalocephaly and developmental delay are common symptoms, although many late-onset patients exhibit bulbar signs and motor disturbances instead. AxD has been modeled in mice by engineering point mutations in the endogenous mouse gene (*Gfap*) orthologous to those found in patients. *Gfap*$^{+/R236H}$ mice spontaneously increase GFAP mRNA and protein, exhibit the pathognomonic Rosenthal fibers, and an increased sensitivity to kainate-induced seizures. There are currently no treatments for AxD, but one possible therapeutic route is to reduce GFAP gene expression. To investigate the potential of ASOs to reduce mouse GFAP expression and disease pathology in AxD models, we screened for ASOs that efficiently targeted mouse GFAP. We chose ASOs that most potently reduced GFAP mRNA in mouse primary cortical cultures and injected them via single intracerebroventricular (ICV) bolus in wild-type mice. Four ASOs significantly reduced GFAP mRNA and protein in brain and spinal cord up to 8 weeks after a single bolus injection. Next, lead ASOs were injected via ICV bolus in *Gfap*$^{+/R236H}$ mice. We found marked suppression (up to 99%) of GFAP mRNA as well as protein in soluble, insoluble, and Rosenthal fiber enriched brain lysate fractions. Strikingly, GFAP ASOs also reversed Rosenthal fiber pathology in mutant mouse brains, and markedly reduced microglial activation/recruitment as indicated by Iba1 staining. Last, ASOs also significantly reduced Nqo1 mRNA in *Gfap*$^{+/R236H}$ mice, indicating a reversal of activation of the Nrf2/ARE antioxidant stress pathway found in this model. In conclusion, we have developed several ASOs targeting mouse GFAP that show great promise in preclinical studies as an approach for treating AxD.

Disclosures: **B. Powers:** A. Employment/Salary (full or part-time): Ionis Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ionis Pharmaceuticals. **T. Hagemann:** None. **S. Wheeler:** None. **C. Mazur:** A. Employment/Salary (full or part-time): Ionis Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ionis Pharmaceuticals. **E. Swayze:** A. Employment/Salary (full or part-time): Ionis Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ionis Pharmaceuticals. **A. Messing:** None.

Poster

692. Demyelinating Disorders: Therapeutics

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Perez Family Research

Title: FTY720 restores brain-derived neurotrophic factor expression in a multiple system atrophy model

Authors: I. SEGURA-ULATE, B. YANG, J. VARGAS-MEDRANO, *R. G. PEREZ; Biomed. Sci., Texas Tech. Univ. Hlth. Sci. Ctr. - El Paso Campus, El Paso, TX

Abstract: Multiple system atrophy (MSA) is a rare and rapidly progressing demyelinating neurodegenerative disorder with no effective treatments. Most MSA patients progress from diagnosis to death in 10 years. MSA pathology is characterized by accumulation of the protein α-synuclein (aSyn) in oligodendroglia cells (OLGs), the myelinating cells of the brain. Importantly, aSyn is not normally expressed in mature OLGs but rather taken up from extracellular aSyn secreted by neurons. aSyn accumulation has deleterious effects in OLGs; including decreased expression of the potent protective molecule, brain derived neurotrophic factor (BDNF). Additionally, increasing BDNF in an MSA mouse model or supplementing an MSA OLG cell model with BDNF reduces their respective MSA dysfunction. Therefore, increasing OLG BDNF may be beneficial for MSA. We first modeled the effects of aSyn neuron-to-OLG transfer on BDNF expression in OLGs, using an *in vitro* aSyn uptake model using the OLG cell line, OLN-93. We incubated cells with recombinant human aSyn for 12 hr and confirmed aSyn uptake by immunoblot and immunocytochemistry. aSyn uptake decreased BDNF expression in OLN-93 cells as determined by qPCR. In an attempt to reverse the BDNF downregulation in response to aSyn, we also evaluated FTY720 (fingolimod, Gilenya), an FDA-approved drug for multiple sclerosis that is known to stimulate BDNF expression in many neural cells. Treatment with FTY720 alone significantly increased BDNF expression in OLN-93 cells, and the loss of BDNF in response to aSyn could be reversed by co-treating with FTY720 + aSyn. As FTY720 has also been shown to inhibit histone deacetylases (HDAC); we evaluated OLN-93 cells for FTY720 effects on histone acetylation, both globally and at BDNF promoters. Immunoblots confirmed

increased histone 3 acetylation (AcH3) levels in response to FTY720. Furthermore, chromatin immunoprecipitation assays showed that AcH3 levels were specifically increased at BDNF promoter 1 in OLN-93 cells treated with FTY720. Here, we report that BDNF expression in OLGs was downregulated by aSyn uptake and that this effect was reversed by FTY720. Furthermore, we found that HDAC inhibition is a potential mechanism by which FTY720 upregulated BDNF in OLN-93 cells. Similar studies with our novel FTY720 analogues, FTY720-Mitoxy and FTY720-C2, also increased neurotrophic factor expression in OLN-93 cells. Thus, FTY720s may be beneficial for MSA, as we are now pre-clinically exploring in an MSA mouse model.

Disclosures: I. Segura-Ulate: None. **B. Yang:** None. **J. Vargas-Medrano:** None. **R.G. Perez:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ruth G. Perez, Patent filed.

Poster

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Support: NIH Grant RO1AG037506

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Title: Mir-146a promotes oligodendrogenesis in the demyelinating central nervous system

Authors: *J. ZHANG[1], Z. ZHANG[1], M. LU[2], S. ELIAS[3], M. CHOPP[1,4];
[1]Neurol. Res., [2]Biostatistics and Res. Epidemiology, [3]Neurol., Henry Ford Hlth. Syst., Detroit, MI; [4]Dept. of Physics, Oakland Univ., Rochester, MI

Abstract: Multiple sclerosis (MS) is a demyelinating autoimmune disease in the central nervous system (CNS) characterized by failure of remyelination. Inefficiency of oligodendrocyte progenitor cell (OPC) differentiation into myelinating oligodendrocytes is a key factor leading to failure of remyelination. MicroRNAs (miRNAs) are mediators of post-transcriptional control of gene expression. We hypothesized that miR-146a, by promoting differentiation of OPCs, will enhance remyelination. Two models of demyelination were employed: 1) experimental autoimmune encephalomyelitis (EAE), an animal model of MS. EAE mice were intravenously injected with miR-146a mimics or controls labeled with fluorescent CY3 once a week for six weeks, starting at day 14 post immunizations (p.i.); and 2) cuprizone (CPZ) diet model, a non-

inflammatory demyelination model. After fed CPZ diet for 5 weeks, mice were continuously infused miR-146a mimics or controls into the corpus callosum for 7 days using an mini osmotic pump. Immunofluorescent staining, ELISA, RT-PCR and Western blot were used to measure the profile of differentiation of OPCs, remyelination and T cell responses, and to investigate the possible underlying mechanisms of miR-146a action. Data revealed that in the EAE model, miR-146a mimics 1) significantly improved neurological outcome from day 27p.i. up to day 90p.i; 2) targeted OPCs in the lesion areas; and 3) significantly increased the number of BrdU+/APC+ cells (newly generated oligodendrocytes), APC+ mature oligodendrocytes and myelin basic protein (MBP) level in the spinal cord; 4) increased Th2 and Treg cells, and decreased Th1 and Th17 cells, as well as their related cytokines, compared with the controls ($p<0.05$), respectively. Furthermore, we confirmed that miR-146a mimics targeted OPCs and promoted OPC differentiation into myelinating oligodendrocytes in the CPZ demyelinating model. Elevation of miR-146a level inhibited the toll-like receptor 2 (TLR2) and interleukin-1 receptor-associated kinase 1 (IRAK1) pathways in the both demyelinating models. Collectively, miR-146a promotes remyelination by indirectly acting on CD4+ T cells, and directly acting on OPCs to enhance functional recovery. This study provides the cellular and molecular basis for the therapeutic effects of miR-146a on remyelination with great potential for treatment of demyelinating disorders.

Disclosures: J. Zhang: None. **Z. Zhang:** None. **M. Lu:** None. **S. Elias:** None. **M. Chopp:** None.

Poster

692. Demyelinating Disorders: Therapeutics

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Topic: B.13. Demyelinating Disorders

Title: MAPC® provides benefit in multiple models of multiple sclerosis and promotes differentiation oligodendrocyte *In vitro*

Authors: *S. A. BUSCH[1], R. CUTRONE[1], B. T. LANG[1], J. A. HAMILTON[1], R. H. MILLER[2], R. W. MAYS[1];
[1]Regenerative Med., Athersys, Inc., Cleveland, OH; [2]George Washington Univ., Washington, DC

Abstract: Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system. Considerable research has targeted the immune system as a mediator of disease progression. Infiltration by a heterogeneous population of immune cells including T cells, B cells

and macrophages, along with increased levels of pro inflammatory cytokines contribute to disease. While current standard of care is effective in preventing progression, comprehensive treatment will include both a reduction in the inflammatory response as well as promotion of remyelination. Multipotent adult progenitor cells (MAPC®) are a well characterized population of bone marrow-derived cells that exhibit immunomodulatory effects in central nervous system injury models such traumatic brain injury, stroke, and spinal cord injury. Here we show that MAPC cell therapy reduces deficits in a mouse model of myelin oligodendrocyte glycoprotein (MOG)-induced experimental allergic encephalomyelitis (EAE). MAPC administered at the time of symptom onset, or two weeks following symptom onset, resulted in statistically significant behavioral improvement compared to vehicle treatment. Luxol fast blue staining demonstrated decreased lesion burden within the spinal cord, and a shift from complete to partial lesions, in MAPC-treated animals compared to controls and electron microscopic analysis provided evidence of remyelination. Additionally, direct injection of MAPC cells into lysolecithin-induced spinal cord lesions resulted in an increased white matter density, suggesting a reduction in demyelination and/or promotion of remyelination. As the immunomodulatory capacity of MAPC therapy is well characterized, we next investigated the ability of MAPC cells and MAPC cell-secreted factors to promote the differentiation of mouse oligodendrocytes. In vitro, we observed a significant increase in the number of myelin basic protein (MBP) and O1 positive oligodendrocytes in the presence of MAPC cells or MAPC cell-conditioned media when compared to control. Depletion of extracellular vesicles from MAPC cell-conditioned media diminished the increase of MBP and O1 positive cells. Our results suggest that MAPC cell-secreted soluble factor(s) and/or extracellular vesicles are capable of driving oligodendrocyte differentiation. We are currently investigating the role of MAPC cell therapy in the regulation of human oligodendrocyte maturation.

Disclosures: **S.A. Busch:** A. Employment/Salary (full or part-time): Athersys, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Athersys, Inc. **R. Cutrone:** A. Employment/Salary (full or part-time): Athersys, Inc. **B.T. Lang:** A. Employment/Salary (full or part-time): Athersys, Inc.. **J.A. Hamilton:** None. **R.H. Miller:** None. **R.W. Mays:** A. Employment/Salary (full or part-time): Athersys, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Athersys, Inc..

Poster

692. Demyelinating Disorders: Therapeutics

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Program#/Poster#: 692.09/N12

Topic: B.13. Demyelinating Disorders

Title: Remyelinating human oligodendrocyte progenitors for regenerative treatment of demyelinating diseases and spinal cord injury

Authors: *M. P. HEFFERAN[1], K. SCHWARTZ[3], T. HAZEL[2], K. JOHE[2], M. LEVY[3]; [1]Neuralstem Inc., San Diego, CA; [2]Neuralstem Inc., Germantown, MD; [3]Johns Hopkins Univ., Baltimore, MD

Abstract: Demyelinating diseases of the central nervous system, including Multiple Sclerosis, Optic Neuritis, and Transverse Myelitis, affect over 400,000 individuals in the United States alone. In addition, progressive demyelination is a pathological component of spinal cord injury, which affects over 200,000 additional individuals. A promising strategy for restoring neurologic function in such populations involves transplantation of expanded precursor cell populations with the capability to remyelinate host axons. However, this approach requires that grafted cells survive in a hostile environment, migrate throughout areas of injury or damage, and produce structurally organized myelin sheath. Here we report results obtained using a clinical grade human neural precursor cell line, NSI-777, that can be expanded extensively ($>10^9$-fold) under chemically defined conditions and can myelinate host axons after demyelination induced by diverse mechanisms including autoimmune processes, chemical injury, or contusion. We tested the ability of NSI-777 to integrate and generate myelin after grafting. NSI-777 injected into corpus callosum and striatum of nude rats survived for at least 6 months and migrated extensively from the graft site, as demonstrated by HuNu staining. Similarly, upon grafting into brains of myelin-deficient *shiverer* pups NSI-777 showed myelination of axon tracts throughout the brain, including corpus callosum, fimbria, and cerebellum, indicated by expression of Olig2 and myelin basic protein (MBP) in HuNu-positive cells. We also evaluated the ability of NSI-777 to engraft in rodent models of demyelination. We first assessed the survival and differentiation of cells in models of focal chemical demyelination and spinal cord contusion. In both cases cells migrated widely from the site of grafting and differentiated into oligodendrocytes in high proportions, reflected by expression of Olig2 and MBP in HuNu-positive cells. We then evaluated the ability of NSI-777 to survive and integrate in an inflammatory environment using a mouse model of multiple sclerosis, experimental autoimmune encephalomyelitis (EAE). A large portion of grafted cells were found within EAE lesions. The cells were Olig2/MBP immunopositive and had fibrous processes with MBP ring-like terminal structures, likely examples of putative myelination by NSI-777. We conclude that NSI-777, a clinical grade line of human neural precursors that can be extensively expanded, can survive, migrate, and differentiate into functional oligodendrocytes in unmyelinated and demyelinated rodent models.

Disclosures: **M.P. Hefferan:** A. Employment/Salary (full or part-time): Neuralstem Inc.. **K. Schwartz:** None. **T. Hazel:** A. Employment/Salary (full or part-time): Neuralstem Inc. **K. Johe:** A. Employment/Salary (full or part-time): Neuralstem Inc.. **M. Levy:** None.

Poster

692. Demyelinating Disorders: Therapeutics

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Topic: B.13. Demyelinating Disorders

Support: NIH (NS34939)

Title: Therapeutic effect of sephin1 on the mouse model of multiple sclerosis, experimental autoimmune encephalomyelitis

Authors: *Y. CHEN, B. POPKO;
Univ. of Chicago, Chicago, IL

Abstract: Multiple sclerosis (MS) is a chronic autoimmune neurological disease most often initiating in young adults. Clinical manifestations are diverse and often disabling, and current immunomodulatory therapies have limited benefit. Immune-mediated oligodendrocyte apoptosis along with subsequent demyelination and axonal degeneration are key attributes of MS progression. Here we explored the therapeutic potential of the integrated stress response (ISR) in providing increased protection to oligodendrocytes and myelin from inflammatory insults. The ISR is a cytoprotective response that is activated by a variety of cytotoxic insults, including inflammation. The ISR is initiated by phosphorylation of eukaryotic translation initiation factor 2 alpha (P-eIF2α) to diminish global protein translation and selectively allow for the synthesis of protective proteins. P-eIF2α is dephosphorylated to terminate the ISR, ensuring the re-initiation of translation . Sephin1 is a recently-identified ISR modifier that inhibits eIF2α dephosphorylation, thereby prolonging the ISR protective response. We demonstrated that sephin1 significantly delayed the onset of clinical symptoms in a mouse model of MS, experimental autoimmune encephalomyelitis. Importantly, we found that the protection provided by sephin1 correlates with reduced oligodendrocytes loss and demyelination in the lumber spinal cord. Moreover, sephin1 suppressed the inflammatory infiltration into the central nervous system. Our results suggest that an oligodendrocyte-protective treatment based on the enhancement of the ISR would likely have significant therapeutic value for MS patients.

Disclosures: Y. Chen: None. **B. Popko:** None.

Poster

693. Demyelinating Disorders: Human and Animal Studies

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Program#/Poster#: 693.01/N14

Topic: B.13. Demyelinating Disorders

Title: Thromboxane A_2 levels are increased in the cerebrospinal fluid of active multiple sclerosis patients.

Authors: *F. MIR, D. BLEMUR, S. A. SADIQ;
Tisch MS Res. Ctr. of NY, New York, NY

Abstract: The prostanoid thromboxane A_2 is an arachidonic acid metabolite generated by the activation of phospholipase A_2 from the cell membrane phospholipid pool. Thromboxane A_2 binding to its receptor (TPR) has been documented to have varied effects in cell types and regulates hemostasis, immune function and inflammation among others. TPR signaling has been shown to function in multiple cell types in the brain. In the current study we investigate its involvement in multiple sclerosis and its animal model experimental autoimmune encephalomyelitis (EAE). Thromboxane A_2 I levels in the cerebrospinal fluid (CSF) were determined by measuring its downstream metabolite thromboxane B_2 using an ELISA from Cayman chemicals, USA. CSF was obtained from controls and MS patients with informed consent under an IRB-approved protocol. We found that thromboxane A_2 levels were significantly increased in the CSF of multiple sclerosis patients (222.6±45.1 pg/ml; n = 75) as compared to controls (108.2 ± 14.3 pg/ml; n = 25). This increase was more pronounced in the CSF of active MS patients. In a parallel EAE study, the expression levels of the thromboxane A_2 receptor (TPR) in the brain and spinal cord were found to be upregulated in the wild-type mice and this increase in TPR levels correlated with the disease course. Furthermore, using an inducible oligodendrocyte specific TPR knockout mouse model, we found that TPR deficiency in oligodendrocytes exacerbates EAE scores in these mice as compared to wild type controls. The upregulation of thromboxane A_2 signaling during active MS and EAE indicates its potential involvement in the pathophysiology and warrants further investigation.

Disclosures: F. Mir: None. **D. blemur:** None. **S.A. Sadiq:** None.

Poster

693. Demyelinating Disorders: Human and Animal Studies

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Program#/Poster#: 693.02/N15

Topic: B.13. Demyelinating Disorders

Title: Cerebrospinal fluid biomarkers of disease phenotype and activity in multiple sclerosis.

Authors: J. TUDDENHAM, *V. K. HARRIS, S. A. SADIQ;
Tisch MS Res. Ctr. of New York, New York, NY

Abstract: Multiple sclerosis (MS) is an inflammatory demyelinating disease, which is associated with progressive disability, cerebellar dysfunction, and cognitive impairment. There is an unmet need for accurate and predictable biomarkers that reflect ongoing disease activity and allow for optimized therapeutic management. In recent years, a number of candidate cerebrospinal fluid (CSF) biomarkers have been discovered through unbiased proteomic approaches. Angiotensinogen has been implicated in regulating the permeability of the blood-brain barrier, and lowered CSF levels of its metabolite, Angiotensin II, have been correlated with MS. Contactin-1 has been identified at reduced levels in the CSF of MS patients. Kallikrein-6 is elevated in the CSF of secondary progressive MS patients. Heightened expression of Superoxide Dismutase (SOD) 1 and 2 has been found in MS gray matter. Increased levels of CSF Osteopontin have been correlated with disease severity and relapse rate. Significant elevation of CSF Fetuin-A has been correlated with active disease. Finally, decreased CSF levels of ITM2B (Bri2) have been associated with cerebellar and cognition impairment. Our objective was to correlate CSF biomarkers with MS subtype, levels of disability, disease activity, clinical phenotype, and treatment. CSF was collected from consenting patients seen at the International Multiple Sclerosis Management Practice, along with demographic and clinical information including MS subtype, treatment, and EDSS. Cerebellar function was assessed by FS2 scale. CSF was immediately centrifuged, and cells were counted using hemacytometer. CSF supernatant was stored at -80°C until use. Angiotensinogen, soluble SOD1, soluble SOD2, Fetuin-A, Osteopontin, Kallikrein-6, Contactin-1, and ITM2B were measured in CSF by Luminex Assay or ELISA. We found a very strong relationship between CSF levels of SOD1, SOD2, Kallikrein-6 and Contactin-1, whereas neither Angiotensinogen nor ITM2B showed a significant correlation with any other biomarker. Moreover, there was a significant correlation between Angiotensinogen and CSF cell count, supporting its involvement in blood brain barrier permeability. A number of biomarkers were found to be significantly reduced in CSF from patients with cerebellar dysfunction, including ITM2B, SOD1, SOD2, Contactin-1 and Kallikrein-6. In addition, ITM2B levels were significantly reduced in the overall pool of patients on disease-modifying treatment compared to untreated patients. Our results identify a panel of CSF biomarkers that reflect neuroinflammation and cerebellar dysfunction associated with MS.

Disclosures: **J. Tuddenham:** None. **V.K. Harris:** None. **S.A. Sadiq:** None.

Poster

693. Demyelinating Disorders: Human and Animal Studies

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Topic: B.13. Demyelinating Disorders

Support: NFB Austria, Project LS 10-32

OptimaMed Austria

Title: Alterations of tryptophan metabolites in patients with multiple sclerosis

Authors: ***B. KEPPLINGER**[1], B. SEDLNITZKY-SEMLER[2], J. REUSS[3], H. BARAN[2]; [1]Karl Landsteiner Res. Institute, Mauer, Mauer-Amstetten, Austria; [2]Karl Landsteiner Res. Institute, Mauer, Mauer-Amstetten, Austria; [3]Neurol., Gen. Hosp., Amstetten, Austria

Abstract: **Background:** Aim of the present study was to investigate changes of tryptophan metabolites along the kynurenine pathway in the cerebrospinal fluid (CSF) and serum of patients with multiple sclerosis (MS).
Patients and Methods: Patients were recruited from the Neurological Department of the General Hospital Amstetten and Neuropsychiatric Hospital Mauer, Austria. Two groups were formed: clinically probable MS (n=63) and clinically definite MS (n=44) according to diagnostic criteria for MS. The amount of L-tryptophan, L-kynurenine, kynurenic acid and anthranilic acid in CSF and serum of patients (n=107) and corresponding control subjects (CO; n=26) were analysed by HPLC method. Correlations of changes were evaluated. Student's t-Test and one-way-ANOVA were applied. The study was performed according to the ethical regulations of the government of Lower Austria.
Results: Kynurenic acid levels were increased significantly in the serum of definite and probable MS (143% and 170% of CO, $p<0.01$) and in the CSF of definite MS (352% of CO; $p<0.01$) but not in CSF of probable MS patients. Anthranilic acid was increased significantly in serum of definite and probable MS (284 % and 291% of CO, $p<0.01$). Ratio: anthranilic acid to L-kynurenine was increased significantly in the serum of probable MS (246% of CO) but not in the CSF; ratio anthranilic acid to kynurenic acid was increased significantly in the serum of probable and of definite MS (212% and 241% of CO, $p<0.05$), but not in the CSF.
Conclusion: Tryptophan metabolism along kynurenine pathway is activated differently in the periphery and in the CNS of definite and probable MS patients. Significant augmentation of kynurenic acid levels was observed in the CSF of definite MS patients, but not of probable MS

patients, suggesting activation of cells, respectively of cell compartments, synthesizing kynurenic acid and specific for MS development. Lowering of glia depressing factor, which we have proposed previously, might be involved in the overactivation of cells respectively, activation of tryptophan metabolism along the kynurenine pathway in MS patients. Study supported by Project LS 10-32, NFB Austria and by Optima*M*ed Austria. Corresponding author: halina.baran@neuro-lab.eu

Disclosures: B. Kepplinger: None. **B. Sedlnitzky-Semler:** None. **J. Reuss:** None. **H. Baran:** None.

Poster

693. Demyelinating Disorders: Human and Animal Studies

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Support: Gemeinnützige Hertie Stiftung

Title: Cellular and molecular mechanisms of spine loss in an animal model of cortical multiple sclerosis

Authors: *A. SCHUMACHER[1,2], M. JAFARI[1,2], T. NEZIRAJ[1,2], S. SCHMIDT[1,2], T. JÜRGENS[3], M. KREUTZFELD[3], D. MERKLER[3,4], M. KERSCHENSTEINER[1,2,5]; [1]Inst. of Clin. Neuroimmunology, LMU Muenchen, Muenchen, Germany; [2]BioMedical Ctr. (BMC), Munich, Germany; [3]Dept. of Pathology and Immunol., Univ. of Geneva, Geneva, Switzerland; [4]Div. of Clin. Pathology, Geneva Univ. Hosp., Geneva, Switzerland; [5]Munich Cluster of Systems Neurol. (SyNergy), Munich, Germany

Abstract: In Multiple Sclerosis (MS), the inflammatory, demyelinating lesions in the white matter have traditionally been attributed to be the main cause of neurological disability. However, over the recent years it has become increasingly clear that the degree of neurological disability shows only limited correlation to the observed numbers and extent of lesions in the white matter. In addition, most MS patients develop also complex and most disabling neuropsychological impairments, such as fatigue, cognitive decline and depression. Indeed refined histopathological and MRI techniques have revealed that MS lesions also appear in the gray matter of MS patients. In previous work, our group was able to show a primary reduction of spine density that occurred both in demyelinated lesions and even in normal-appearing areas of MS cortex. We could now reproduce the widespread spine loss seen in the MS tissue in an animal model of cortical MS pathology. Using transgenic reporter mouse lines, we then

characterized the distribution and contribution of infiltrating mononuclear phagocytes and activated resident microglia. In vivo 2-Photon imaging of neurons, which we virally labeled with the genetically-encoded calcium indicator protein Twitch-2B, further allows us to monitor neuronal calcium levels in relation to spine and dendrite pathology. By studying the interactions between mononuclear phagocytes and neurons and the resulting changes of intracellular calcium levels over time, we aim to resolve the mechanisms underlying inflammatory spine loss and thereby establish the basis for the development of new treatment strategies that counteract cortical MS symptoms.

Disclosures: A. Schumacher: None. **M. Jafari:** None. **T. Neziraj:** None. **S. Schmidt:** None. **T. Jürgens:** None. **M. Kreutzfeld:** None. **D. Merkler:** None. **M. Kerschensteiner:** None.

Poster

693. Demyelinating Disorders: Human and Animal Studies

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 693.05/N18

Topic: B.13. Demyelinating Disorders

Support: Gemeinnützige Hertie Stiftung

Title: Widespread spine loss in cortical multiple sclerosis and its model

Authors: *M. JAFARI[1,2], A. SCHUMACHER[1,2], T. JÜRGENS[3], M. KREUTZFELD[3], S. SCHMIDT[1,2], T. NEZIRAJ[1,2], D. MERKLER[3,4], M. KERSCHENSTEINER[1,2,5];
[1]Inst. of Clin. Neuroimmunology, LMU Munich, Muenchen, Germany; [2]Biomed. Ctr. (BMC), LMU Munich, Munich, Germany; [3]Dept. of Pathology and Immunol., Univ. of Geneva, Geneva, Switzerland; [4]Div. of Clin. Pathology, Geneva Univ. Hosp., Geneva, Switzerland; [5]Munich Cluster of Systems Neurol. (SyNergy), Munich, Germany

Abstract: Multiple Sclerosis (MS) is an inflammatory disease of the CNS and one of the major causes of neurological disability. Damage to cortical grey matter is reported from early stages of the disease increase with disease duration and are associated with physical disability and cognitive impairment. To better understand how grey matter pathology is initiated we recently reconstructed cortical projection neurons in autopsy tissue from multiple sclerosis patients. Our analysis revealed a widespread and pronounced loss of dendritic spines that was present both in demyelinated and normal-appearing areas of the MS cortex. To further investigate the mechanisms involved in the elimination of dendritic spines, we have now established a mouse model of cortical MS pathology that is induced by the stereotactic cortical injection of pro-

inflammatory cytokines in mice immunized with the myelin oligodendrocyte glycoprotein. In this model cortical demyelination was primarily detected in subpial areas reminiscent of the cortical lesion type III pathology observed in human MS samples. Using high resolution confocal imaging, we studied the apical dendrites of transgenically labelled cortical layer 5 pyramidal neurons and detected a widespread loss of spines both ipsi- and contralateral to the injection site in all cortical layers mimicking the pathology we previously reported in human tissue and supporting an inflammatory pathogenesis of the synaptic pathology.

Disclosures: **M. Jafari:** None. **A. Schumacher:** None. **T. Jürgens:** None. **M. Kreutzfeld:** None. **S. Schmidt:** None. **T. Neziraj:** None. **D. Merkler:** None. **M. Kerschensteiner:** None.

Poster

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Topic: B.13. Demyelinating Disorders

Title: Tracking axonal conduction changes in multiple sensory modalities in a mouse model of oligodendrocyte loss.

Authors: ***B. J. FARLEY**, E. MOROZOVA, J. AMACKER, B. WANG, B. D. HARVEY, D. GIANNI, B. T. WIPKE, M. HAJOS;
Biogen, Cambridge, MA

Abstract: In multiple sclerosis patients, sensory evoked potentials can serve as a biomarker of functional neurotransmission in central nervous system pathways. However, since changes in myelination, inflammation, and axonal degeneration can occur together in the disease, it is challenging to know how any one process contributes to evoked potential changes. In the Rosa26-DTA; PLP-Cre ("DTA") mouse model of induced oligodendrocyte ablation, demyelination and subsequent remyelination have been reported without extensive axonal degeneration or inflammation (Traka et al., *Brain*, 2010). We monitored visual, auditory, and somatosensory evoked potentials (VEP, AEP, and SSEP) longitudinally in DTA mice with electrodes chronically implanted over the respective sensory cortical areas. Beginning 4 weeks after model induction (by tamoxifen administration), we observed an increase in AEP latency ($p<0.0001$, 2-way ANOVA) and SSEP latency ($p<0.001$, 2-way ANOVA) in tamoxifen-injected mice relative to vehicle-injected (control) mice. VEP latencies, in contrast, were unaffected at the tamoxifen dose employed. Continued monitoring of AEPs revealed that the latency increase was maximal 8 weeks after model induction, when the peak latency was 35% longer in tamoxifen-treated compared to control mice (23.7 +/- 0.4 vs. 17.6 +/- 0.2 ms). Thereafter, AEP latencies

gradually recovered, being only 11% longer in tamoxifen-induced mice by week 12 (19.9 +/- 0.3 vs. 18.0 +/- 0.3 ms). The timecourse of the AEP latency increase and its subsequent recovery suggests that it may reflect changes in CNS myelination (demyelination followed by remyelination) previously reported in this model. To assess peripheral neurotransmission, we also measured the compound muscle action potential (CMAP) in the mice. Pronounced changes in CMAP latency ($p<0.0001$, 2-way ANOVA) as well as amplitude ($p<0.0001$, 2-way ANOVA) were observed in tamoxifen-induced mice. However, these changes began and recovered with an accelerated timecourse relative to the AEP and SSEP changes, suggesting that the mechanisms driving peripheral changes are distinct from those driving the AEP and SSEP changes. To further test the hypothesis that AEP and SSEP latency changes reflect CNS demyelination and remyelination, and support their use as a translational biomarker of these processes, histological examination of relevant neural pathways are being performed.

Disclosures: **B.J. Farley:** A. Employment/Salary (full or part-time): Biogen. **E. Morozova:** A. Employment/Salary (full or part-time): Biogen. **J. Amacker:** A. Employment/Salary (full or part-time): Biogen. **B. Wang:** A. Employment/Salary (full or part-time): Biogen. **B.D. Harvey:** A. Employment/Salary (full or part-time): Biogen. **D. Gianni:** A. Employment/Salary (full or part-time): Biogen. **B.T. Wipke:** A. Employment/Salary (full or part-time): Biogen. **M. Hajos:** A. Employment/Salary (full or part-time): Biogen.

Poster

693. Demyelinating Disorders: Human and Animal Studies

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 693.07/O2

Topic: B.13. Demyelinating Disorders

Support: Brians Hope

The Myelin Project

Title: Human macrophages in Adrenoleukodystrophy show phenotypic specific cytokine and glutamate response to very long chain fatty acid stimulation

Authors: ***B. R. TURK**[1], C. TIFFANY[3], B. E. THEISEN[1], M. ROSEN[2], C. NEMETH[3], J. MARX[3], R. O. JONES[3], P. WATKINS[2], A. B. MOSER[3], S. KANNAN[4], A. FATEMI[3]; [1]Neuroscience, Kennedy Krieger Inst., [2]Johns Hopkins Univ., Baltimore, MD; [3]Kennedy Krieger Inst., Baltimore, MD; [4]Johns Hopkins Sch. of Med., Baltimore, MD

Abstract: Adrenoleukodystrophy is an X-linked peroxisomal disorder due to ABCD1 mutation and very long chain fatty acid (VLCFA) accumulation. Metabolic and oxidative stress are proposed central effectors of general ALD cell pathology, while microglia activation in brain has been indicated as a mediator of deadly cerebral disease. We attempted to create indiviual macrophage models from each ALD patient and phenotype in order to determine reaction to stimulation with VLCFAs C24:0 and C26:0.Peripheral Blood Mononuclear Cell (PBMC) culture was derived froml consented ALD patients (n=21) and healthy controls (n=5) at Kennedy Krieger Institute, then differentiated into M1 polarised macrophages over 7 days. Monocyte and macrophage cultures were stimulated with VLCFA and lipopolysaccharide (LPS) at multiple timepoints and doses.Monocytes and Macrophages showed dose dependant response to LPS stimulation in secretion of pro-inflammatory cytokines IL-6 and TNFalpha. VLCFA stimulation showed high fold over base increase in cerebral ALD phenotype, but not heterozygote female carrier (Het) or adrenomyeloneuropathy (AMN) of TNF alpha and Glutamate.
A phenotypic specific PBMC in-vitro cytokine response may indicate either a cytokine profile shift after cerebral disease onset, or discernible prognistic parameter before cerebral onset. Additionally, this novel in-vitro model may serve in testing pharmacological agents in reducing pro-inflammatory cytokine production or other oxidative stress parameters in ALD.

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Poster

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Program#/Poster#: 693.08/O3

Topic: B.13. Demyelinating Disorders

Support: Supported by Research Fellowship Award from Questcor/Mallinckrodt Pharmaceuticals

Title: Enlarged perivascular spaces are not associated with disease worsening in patients with relapsing-remitting multiple sclerosis

Authors: *M. CAVALLARI[1], S. EGOROVA[2], B. C. HEALY[2], M. PALOTAI[1], J. PRIETO[1], M. POLGAR-TURCSANYI[2], M. ANDERSON[2], B. GLANZ[2], T. CHITNIS[2], C. R. G. GUTTMANN[1];
[1]Radiology - Ctr. for Neurolog. Imaging, [2]Brigham and Women's Hosp., boston, MA

Abstract: Enlarged perivascular spaces (EPVS) have been recently proposed as a means to investigate abnormalities of the brain glymphatic system, which is considered to have a role in the clearance of neurotoxic metabolites. Multiple Sclerosis (MS) patients showed higher EPVS count and volume compared to healthy individuals in a few recent case-control MRI studies. EPVS have also been associated with relapses and brain atrophy in MS patients, thus supporting the role of impaired glymphatic clearance of neurotoxic metabolites in neuroinflammatory and neurodegenerative features of the disease.
In this study we investigated the relevance of EPVS to clinical disease worsening in MS. We retrospectively selected from the CLIMB (Comprehensive Longitudinal Investigation of Multiple Sclerosis at Brigham and Women's Hospital) study cohort relapsing-remitting MS patients (RRMS) who converted to confirmed (≥2 years) Expanded Disability Status Scale (EDSS) score ≥3 within a follow-up period ≥3 years. We contrasted the baseline EPVS score of 30 converters, obtained from 1.5T dual-echo proton density/T2-weighted MRI images before conversion to EDSS ≥3, with that of 30 non-converter RRMS patients matched for baseline characteristics (age, sex, disease duration). We also investigated the predictive role of EPVS towards magnetic resonance imaging (MRI) measures of accrual of brain atrophy and T2 lesion volume (T2LV) after the EPVS assessment (median MRI follow-up: 2 years, range: 0-11 years). Baseline EPVS scores were not significantly different between converters and non-converters to EDSS ≥3, and were not significantly associated with accrual of brain atrophy or T2LV.
In this pilot study of a well characterized cohort of MS patients followed longitudinally for up to 11 years we did not find significant associations between EPVS and clinical or radiological indicators of disease worsening. Since the small sample size, as well as the suboptimal MRI data characteristics for visualization of EPVS might have limited our ability to detect a significant effect, future studies are warranted to investigate the potential clinical relevance of EPVS in MS.

Disclosures: **M. Cavallari:** A. Employment/Salary (full or part-time): Center for Neurological Imaging, Department of Radiology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA. 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; Supported by Research Fellowship Award from Questcor/Mallinckrodt Pharmaceuticals. **S. Egorova:** 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; receives research support from Merck Serono, Novartis and Foundation for Neurologic Diseases Boston. **B.C. Healy:** 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; receives research support from Merck Serono, Novartis and Genzyme. **M. Palotai:** 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; supported by the MS International Federation McDonald Fellowship Award. **J. Prieto:** 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; Supported by Research Fellowship Award from Questcor/Mallinckrodt Pharmaceuticals. **M. Polgar-Turcsanyi:** None. **M. Anderson:** None. **B. Glanz:** 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; receives research support from Merck Serono and Verily Life Science. **T. Chitnis:** None. **C.R.G. Guttmann:** None.

Poster

693. Demyelinating Disorders: Human and Animal Studies

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Topic: B.13. Demyelinating Disorders

Support: Quinnipiac University

Title: Pathogenesis of experimental autoimmune encephalomyelitis is modulated by glutamatergic signaling and inflammatory cytokines in the hippocampus and prefrontal cortex

Authors: R. ROTOLO[1], J. DEMURO[2], G. DRUMMOND[1], J. WOOD[3], C. LITTLE[3], L. TELISKA[3], T. STRANGE[3], J. VIDAL[3], L. FRUEHAUF[3], A. BARBER[3], A. WOLF[3], J. BLAKE[3], L. JOHNS[1], *A. J. BETZ[3];
[1]Hlth. Sci., [2]Mol. and Cell. Biol., [3]Psychology, Quinnipiac Univ., Hamden, CT

Abstract: There is a great demand for therapeutic advancements with respect to autoimmune disorders such as Multiple Sclerosis (MS). Experimental Autoimmune Encephalomyelitis (EAE) is an inflammatory disease of the central nervous system with neurological and pathological similarities to MS. In this study, EAE-exposed C57BL/6 mice were used to characterize the cognitive and physical impairments and to establish the molecular markers associated with EAE pathogenesis. A group of mice was pre-trained on behavioral tasks (BEH) while being calorie restricted (CR) prior to myelin oligodendrocyte glycoprotein (MOG) exposure, inducing EAE. A combination of CR and BEH resulted in a delay of symptom onset and reduced clinical severity, as indicated by the daily progression of ascending tail paralysis. In addition, nociceptive sensitivity was preserved in mice exposed to EAE + CR and CR + BEH, similar to controls, whereas EAE-exposed mice demonstrated a greater latency to perceive a painful stimulus at the peak of disease severity. These results suggest that CR and BEH may play a modulatory role in the development of EAE. In a second experiment, mice were treated with riluzole, a glutamate release inhibitor, to assess EAE pathology. Since glutamate excitotoxicity has been found in MS lesions, we proposed that clinical severity would be regulated by glutamate availability in the

central nervous system. The histopathological similarities of EAE and MS reveal the presence of inflammation and immunological mechanisms complementary to disease initiation and progression. The presence of glutamate, infiltrating immune cells, and immune-regulatory cytokines, can influence the pathology of EAE by providing insight as to which cytokines are expressed at heightened disease states. Taken together, these data reveal distinct molecular markers that are associated with ameliorated symptoms. More specifically, alterations in prefrontal cortex (PFC) cytokine expression of GM-CSF, IL-1α, MIG, RANTES, and TNFα, and hippocampal expression of GFAP, TRAF3, Foxp3, and NF-κB, may provide insight as to the molecular mechanisms responsible for hallmark EAE pathogenesis and possible therapeutic remedies.

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Poster

693. Demyelinating Disorders: Human and Animal Studies

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Title: Adaptive immunity drives remyelination failure or success in multiple sclerosis

Authors: ***V. ZUJOVIC**[1], C. SANSON[1], M. EL BEHI[1], C. BACHELIN[1], L. GUILLOT-NOËL[1], N. SARRAZIN[1], J. FRANSSON[1,2], E. MAILLART[2,3], B. STANKOFF[4], V. GUILLEMOT[1], H. ABDI[3,4], I. REBEIX[1], B. FONTAINE[1];
[1]Sorbonne-universités-Upmc 06, INSERM, CNRS, UMR IC, Paris, France; [2]Neurol. Service, Assistance Publique-Hôpitaux de Paris, Hôpital St. Antoine-HUEP, Paris, France; [3]Sch. of Brain

and Behavioral Sci., The Univ. of Texas, Dallas, TX; [4]Neurol. department, Assistance Publique-Hôpitaux de Paris/Pitié Salpétrière Univ. Hosp., Paris, France

Abstract: One of the major challenges in multiple sclerosis (MS) is to comprehend disease severity progression. The recently demonstrated correlation between disease severity and remyelination emphasizes the importance of identifying factors leading to a favorable outcome. Why remyelination fails or succeeds in MS patients remains largely unknown, mainly because remyelination has never been addressed within a humanized pathological context. Therefore, we developed a new paradigm mimicking MS lesion by grafting human lymphocytes (LT) in the demyelinated lesion of Nude mice spinal cord. We show that LT play a major role in remyelination whose efficacy is significantly decreased in mice grafted with MS LT compared to those grafted with healthy donor LT. Mechanistically, we demonstrated *in vitro* that LT-derived mediators influenced oligodendrocyte precursor cells (OPC) differentiation through a crosstalk with microglial cells. Among mice grafted with LT from different patient, we observed diverse remyelination patterns reproducing for the first time the heterogeneity observed in MS patients. Comparing LT secretory profile from patients exhibiting high and low remyelination ability, we identified novel molecules involved in OPC differentiation and validated one as a target to improve remyelination. In summary, MS LT do exhibit intrinsic capacities to coordinate myelin repair, an effect implying that remyelination heterogeneity arises in part from patients specific LT activation profile. Furthermore, our data strongly suggest that studying MS patients who display high remyelination capacities might provide new pro-regenerative strategies.

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Poster

693. Demyelinating Disorders: Human and Animal Studies

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Topic: B.13. Demyelinating Disorders

Support: MS Society Canada

Title: The effects of ambient temperatures and exercise modality on corticospinal excitability of the soleus in persons with multiple sclerosis

Authors: *G. GROVER[1], D. T. G. PHILPOTT[1], E. M. WALLACK[2], L. P. KELLY[2], A. J. DEVASAHAYAM[2], M. C. KIRKALND[2], A. R. CHAVES[2], M. PLOUGHMAN[1,2], K. E.

POWER[1,2], D. C. BUTTON[1,2];
[1]Sch. of Human Kinetics and Recreation, [2]Fac. of Med., Mem. Univ. of Newfoundland, St. John's, NL, Canada

Abstract: Corticospinal excitability (CSE) in persons with multiple sclerosis (PwMS) is reduced, likely contributing to fatigue and limiting one's ability to engage in physical activity. Given that fatigue is task- and temperature-dependent, the purpose of the study was to determine how a combination of temperature (cool vs. room) and exercise (treadmill vs. recumbent NuStep) affects CSE in PwMS. We hypothesized that CSE, irrespective of exercise modality, would be higher following exercise in the cool environment than in a room temperature due to lower levels of fatigue. Fourteen heat sensitive MS patients (10 Females), 49.28 ± 13.56 years of age with relapsing remitting MS and baseline expanded disability status scores ranging from 3.4 ± 2.37 participated in the study. Transcranial magnetic stimulation (TMS) elicited motor evoked potentials (MEPs) and were assessed prior to and following aerobic exercise interventions at 65% of VO_{2max}. Tibial nerve stimulation elicited maximal muscle compound action potential (M_{max}). Measurements were taken from the soleus muscle of the weakest limb both at rest and during a torque equivalent to 10% of maximal voluntary contraction (MVC). Participants attended four randomized experimental sessions including temperature ((Cool (16°C) and Room (21°C)) and exercise modality ((Treadmill (T) and NuStep (N)). Therefore, the experimental sessions were T in cool (TC), T in room (TR), N in cool (NC) and N in room (NR). MEP amplitudes were made relative to M_{max} amplitudes for analysis. MVC torque of the plantar flexors did not differ pre- and post-condition (TC, p = .09; TR, p = .11; NC, p = .125; NR, p = .327). MEPs recorded from the soleus during rest were elicited in 5/14 and 6/14 patients, pre- and post-exercise, respectively. MEPs recorded at 10% of MVC occurred in 6/14 and 7/14 patients, pre- and post-exercise respectively. CSE was increased in room temperature conditions post-exercise (MEP amplitudes as a percentage of M_{max} in C, 4.78 ± 0.9%; R, 23.19 ± 3.15%; p = .026) and increased following exercise using the NuStep (MEP amplitudes as a percentage of M_{max} in N, 21.84 ± 3.93; T, 6.12 ± 1.21%; p = .029). The lack of decrease in MVC suggests that the exercise interventions employed in this study did not induce fatigue of the plantar flexors. Temperature and the exercise modality influence CSE such that exercising in cool temperature and exercising on a NuStep enhance post-exercise CSE in PwMS.

Disclosures: G. Grover: None. **D.T.G. Philpott:** None. **E.M. Wallack:** None. **L.P. Kelly:** None. **A.J. Devasahayam:** None. **M.C. Kirkalnd:** None. **A.R. Chaves:** None. **M. Ploughman:** None. **K.E. Power:** None. **D.C. Button:** None.

Poster

693. Demyelinating Disorders: Human and Animal Studies

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Topic: B.13. Demyelinating Disorders

Support: Novartis Pharma, AG

Title: Astrocytic activity modulated by S1P signaling in experimental autoimmune encephalomyelitis (EAE) revealed in a c-Fos reporter mouse

Authors: *D. JONNALAGADDA[1], A. GROVES[2], Y. KIHARA[3], J. CHUN[3];
[1]Mol. and Cell. Neurosci., The Scripps Res. Institute, La Jolla, CA, La Jolla, CA; [2]Sch. of Med., UCSD, La Jolla, CA; [3]Dept. of Mol. and Cell. Neurosci., The Scripps Res. Inst., La Jolla, CA

Abstract: Background. FTY720 (fingolimod, Gilenya) is an FDA-approved, highly effective oral drug for treating MS, showing efficacy in both EAE (a mouse model of human MS) and MS. FTY720 crosses the blood brain barrier and becomes phosphorylated to produce FTY720-P that is a chemical analog of sphingosine 1-phosphate (S1P). S1P signaling is altered in MS patients. FTY720-P binds to S1P receptors ($S1P_{1,3-5}$) expressed on various cell types. FTY720's efficacy in EAE is largely dependent on specific binding of FTY720-P to $S1P_1$ expressed on astrocytes, which is required for drug efficacy in EAE. It remains unknown how this astrocyte mechanism of action produces efficacies, which we examine here. **Methods and Results:** A transgenic mouse line with a tetracycline-suppressible *cis* element (tTA) and a c-Fos driven nuclear GFP-histone fusion protein was crossed with an astrocyte specific, conditional $S1P_1$-null mouse line. Monophasic EAE was induced, then tTA activated upon onset of EAE signs, allowing c-Fos activated cells to express a semi-permanent GFP signal. Astrocytes are the predominantly activated cell-type. The extent of activation correlated with disease severity as demonstrated by flow cytometry. Peripheral activation of astrocytes was visualized in the intact lower spinal cord using tissue clearance techniques. GFP-positive EAE-activated astrocytes were isolated by FACS and processed for RNAseq to identify transcriptional changes that revealed clear differences among the untreated, FTY720 treated, and astrocyte specific $S1P_1$ null astrocytes. These differences are being further interrogated. **Conclusions:** The c-Fos reporter mouse identifies a sub-set of astrocyte population that gets activated upon EAE sign onset. The extent of activation correlates with clinical severity. FTY720 administration and astrocyte-targeted $S1P_1$ removal reduces the number of activated astrocytes, indicating role of $S1P_1$ signaling in ameliorating clinical score. Three-dimensional examination of the lower spinal cord during the course of EAE development shows an increase in activated astrocytes in lesions, as well as along the periphery of the spinal cord. Distinct transcriptional changes during EAE were identified.

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Poster

693. Demyelinating Disorders: Human and Animal Studies

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Topic: B.13. Demyelinating Disorders

Title: Cuprizone model - correlation between fine motor performance and white matter changes

Authors: K. LEHTIMÄKI, *S. KIM, A.-M. ZAINANA, A. NURMI;
Charles River Discovery, Kuopio, Finland

Abstract: The cuprizone exposure has been used to model a wide range of pathological and behavioral deficits endpoints that recapitulate demyelinating diseases and multiple sclerosis (MS). We and others have described the behavioral, pathological and immunological phenotype of mouse cuprizone model at different stages during and after cuprizone challenge. Besides traditional behavioral tools, we have applied kinematic analysis to understand more in detail affected motor behavior due relatively mild phenotype during and after cuprizone exposure. In addition, we have also evaluated the extent of the demyelination, both with classical histological/immunohistochemical tools, but also by applying non-invasive imaging. DTI-MRI revealed progressive demyelination in the major white matter structures during cuprizone exposure. Given that many of the behavioral end-points demonstrate recovery following discontinuation of cuprizone treatment, we wanted in the present study evaluate correlation between white matter changes and fine motor behavior more in detail, during and after cuprizone challenge. C57Bl/6 female mice were subjected to oral cuprizone exposure or vehicle for 6 weeks, followed by 3 weeks of recovery without cuprizone. Diffusion tensor magnetic resonance imaging (DTI-MRI) was performed at 3 and 6 weeks while on cuprizone, and on 7, 8 and 9 weeks after removal of cuprizone challenge. Kinematic gait analysis of the cuprizone and vehicle treated mice were performed on weeks 3, 6 and 9 during the study to correlate white matter changes with fine motor gait changes. We found that there are significant correlations between gait parameter changes and white matter changes. Strongest correlations between gait parameters and white matter changes were seen in subregions of corpus callosum (genu, body and splenium) and external capsule, whereas other white matter rich regions did not show either robust demyelination nor did show strong correlation between gait parameters and the structure analyzed. Taken together, data presented in this presentation provide further insight to cuprizone model demyelination, but also to kinetics of remyelination in the model after elimination of the

cuprizone challenge. We also present how different phases demyelination and remyelination are correlated with fine motor performance.

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Poster

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Program#/Poster#: 693.14/O9

Topic: B.13. Demyelinating Disorders

Support: T32HD007414-22

Title: Multiple measures of corticospinal excitability account for clinical disability in transverse myelitis

Authors: *N. F. WYMBS[1,3], M. LEVY[2], K. SCHWARTZ[2], G. CANTARERO[1,4], M. A. MEALY[2], D. BECKER[2], C. A. PARDO[2], P. A. CELNIK[1], K. M. ZACKOWSKI[1,3]; [1]Physical Med. and Rehabil., [2]Neurol., Johns Hopkins Univ., Baltimore, MD; [3]Kennedy Krieger Inst., Baltimore, MD; [4]Walter Reed Army Inst. of Res., Silver Spring, MD

Abstract: Transverse myelitis (TM) is a neurological disorder caused by inflammation of both sides of at least one segment of the spinal cord. Damage often leads to persistent disability of upper and lower limbs, which can result in the substantial impairment of activities in daily life. Little is known regarding the underlying motor neurophysiology and its relationship to clinical disability. Here, we used transcranial magnetic stimulation (TMS) to study corticospinal excitability in participants with TM with the goal of utilizing physiological evidence as a biomarker for disability. Corticospinal excitability of first dorsal interosseous (FDI) and tibialis anterior (TA) muscles, localized to the subject-identified less-impaired side of the body, were assessed using motor evoked potential (MEP) following single-pulse stimulation in 19 individuals with TM and 10 aged-matched healthy controls. Corticospinal excitability was assessed in both relaxed and active muscle states, with stimulation intensity set to generate MEP amplitude of 1 mV (S1mV). In addition, input-output curves (IO-curve) of resting FDI were collected. Relative to controls, we found that TM participants had increased MEP onset latency for both resting FDI (n_{TM} = 19) and TA (n_{TM} = 12). This effect of increased MEP latency was observed for multiple measures (S1mv and IO-curve samples) for TM participants. Further, resting MEP latency (S1mV) was correlated with disability (EDSS) for both FDI (R^2 = 0.27, p = 0.02; Rho = 0.54, p = 0.02) and TA (R^2 = 0.30, p = 0.06; Rho = 0.72, p = 0.008). Using stepwise regression with frontward selection of inputs, TM disability was supported by a 2-variable model

($R^2 = 0.77$, $p < 0.0005$) first by selection of resting FDI latency (S1mV) and second, by lesion location (upper segment as cervical or thoracic). All additional model variables, including active S1mV for FDI and TM, as well as demographic factors, did not reach selection criteria significance ($p < 0.05$). Because we did not observe differences in MEP amplitude for any measure between control and TM groups, our results suggest similar activation of corticospinal populations. Together, these results suggest that demyelination of the spinal cord is central to TM disability. Moreover, these findings indicate that corticospinal physiology may aid in the clinical evaluation and rehabilitation of people with TM.

Disclosures: **N.F. Wymbs:** None. **M. Levy:** 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. **K. Schwartz:** None. **G. Cantarero:** None. **M.A. Mealy:** None. **D. Becker:** None. **C.A. Pardo:** 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. **P.A. Celnik:** None. **K.M. Zackowski:** 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.

Poster

693. Demyelinating Disorders: Human and Animal Studies

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 693.15/O10

Topic: B.13. Demyelinating Disorders

Support: Marmara University BAP SAG-A-130511-0130

Title: Effects of electromagnetic waves emitted by cell phones on rat optic and trigeminal nerve

Authors: *O. E. TOK[1], D. AKAKIN[2], N. DAGBASI[2], D. ANIL[2], A. AKAKIN[3], S. SIRVANCI[2], F. ERCAN[2];
[1]Bezmialem Vakif Univ., Istanbul, Turkey; [2]Histology and Embryology, Marmara Univ., Istanbul, Turkey; [3]Neurosurg., Bahcesehir Univ., ISTANBUL, Turkey

Abstract: Being increasingly used the cell phones have payed attention for their potential effects on living cells. Thermal and electromagnetic wave (EMW) effects of cell phones have been reported on the cranial nerves beside many other different organs. However, their effects on the

optic and trigeminal nerves have not been reported. In addition, because the thickness of the skull is very thin, rate of absorption of EMW in children are higher than adults. Therefore, we aimed to study the effects of EMW emitted from cell phones on the optic and trigeminal nerves beginning from the fetal period.
Male Wistar-albino rats (5 groups; 6 animals in each group) were used in this study. Cell phone was either on call to another cell phone of similar SAR (specific absorption rate) value or on stand-by mode (2 hours/day). Rats in Stand-by/EMD group were exposed to EMW of cell phones starting from intrauterine 14^{th} day to postnatal 60^{th} day. Rats in Stand-by fetal/EMD fetal group were exposed to EMW of cell phones starting from intrauterine 14^{th} day to birth. Rats in the control group were not exposed to cell phone. At the end of the second month, optic and trigeminal nerve samples obtained from rats perfused with 4% paraformaldehyde were processed for electron microscopic evaluation. Semi-thin sections were stained by Toluidine blue and photographed by light microscopy. Optic and trigeminal nerve areas were measured and the myelinated axons were counted in four photographs (100x magnification) from each animal using Image J Programme. Thin sections were evaluated and photographed by transmission electron microscopy.
The optic and trigeminal nerve areas showed no significant difference between experimental groups. The number of myelinated axons were observed significantly decreased in the Stand-by and EMW groups in optic nerve and the EMW group in trigeminal nerve compared to control group. Electron microscopic examination showed normal axon morphology in control groups of optic and trigeminal nerve. For optic nerves, myelin sheath disturbance and degenerated neurofilaments in axoplasma were found in EMW group. In addition, thickened myelin sheath was evident in EMW fetal group. Myelin sheath disturbance in stand-by, EMW fetal and EMW groups and vacuole formation in axoplasma in EMD group were seen in trigeminal nerves.
The findings of this study shows that the EMW emitted by cell phones may have effect on optic and trigeminal nerve from fetal period until adulthood and may create potentially serious consequences in the long-term use. Our study is important to show the effects of the EMWs associated with the exposure cell phones on the cranial nerves at intrauterine life, childhood and adolescence.

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Poster

694. In Vitro and In Vivo Analysis of Tau Pathology

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 694.01/O11

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Plexin-A4 regulates secretion and expression of tau in Alzheimer's disease mouse model

Authors: *J. YANG[1], W. LEE[1], A. KIM[1], J.-C. PARK[1], H. CHOI[1], H. CHOI[1], E. HWANG[2], I. MOOK-JUNG[1];
[1]Seoul Natl. Univ. Col. of Med., Seoul, Korea, Republic of; [2]Ctr. for Functional Connectomics, Korea Inst. of Sci. and Technol. (KIST), Seoul, Korea, Republic of

Abstract: Recent genome-wide association studies identified several single nucleotide polymorphisms in *PLXNA4* of late-onset Alzheimer's disease (AD). It was reported that *PLXNA4* transcript 1 (TS1) mRNA was increased in late AD and was involved in hyperphosphorylation of tau stimulated by SEMA3A. Plexins are receptors or coreptors of SEMA family and have been well known for their functions in neurodevelopment such as axon guidance, lamination of the hippocampus, cytoskeletal regulation of growth cones and recruitment of receptor subunits along the axon. However, it is unknown that how Plexin-A4 can lead to pathophysiology of tau in AD. Here I show whether Plexin-A4 regulates secretion and expression of tau in an isoform dependent manner. In addition, when Plexin-A4 was knock-downed in mouse neurons, pTau was decreased. Finally, AAV-Plexin-A4 shRNA-GFP was used to reveal the role of Plexin-A4 *in vivo*.

Disclosures: J. Yang: None. **W. Lee:** None. **A. Kim:** None. **J. Park:** None. **H. Choi:** None. **H. Choi:** None. **E. Hwang:** None. **I. Mook-Jung:** None.

Poster

694. In Vitro and In Vivo Analysis of Tau Pathology

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 694.02/O12

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: IWT140773

Title: Characterization of pathological Tau species from mouse Tauopathy models and AD brain

Authors: *A. MARREIRO[1,2], K. VAN KOLEN[1], W. BRUINZEEL[1], D. VAN DAM[3], P. P. DE DEYN[3], L. TEMMERMAN[2], G. DEPUYDT[2], L. SCHOOFS[2], M. H. MERCKEN[1];
[1]Janssen Pharmaceutica, Beerse, Belgium; [2]Div. of Animal Physiol. and Neurobio., Katholieke Univ. Leuven, Leuven, Belgium; [3]Dept. of Biomed. Sci. Inst. Born-Bunge, Univ. of Antwerp, Antwerp, Belgium

Abstract: Objectives: Although several studies demonstrate that Tau filaments have seeding potential on both cellular and *in vivo* models, the information on the size of these aggregates has

been ambiguous [1,2]. The aim of this study is to characterize Tau species by their molecular weight and other molecular properties, such as phosphorylation status.
Methods: Aggregates were isolated from *post mortem* AD and transgenic mice (P301S) brain tissue using different extraction procedures. Biochemical characterization was done by Western blotting (reducing and non-reducing), sucrose gradient, size exclusion chromatography and aggregate specific immuno-assays. Structural differences between Tau seeds were analyzed by immuno-EM. Seeding efficiency was then assessed by a FRET cellular model [3].
Results: It has been shown that spinal cord and brainstem total extracts from P301S mice trigger Tau aggregation in HEK293 cells expressing K18 FRET sensors [3]. First, it is investigated which subfraction of such total extracts is the most potent for seeding in this cellular model. By differential centrifugation and detergent treatment, different fractions from aging mice and AD *post mortem* brain tissue are used for aggregation induction and biochemical analysis. In the P301S mouse brain extracts we observed an age-dependent correlation between Tau aggregate load and seeding efficiency. We also observed that both total extract and insoluble fraction from *post mortem* AD brain tissue can induce aggregation in this cellular model. Molecular weight separation both by sucrose gradient and SEC is performed to identify which species show seeding efficiency.
Conclusions: This study further elucidates the molecular properties, regarding molecular weight, phosphorylation status and seeding efficiency of high molecular weight Tau species. This information is essential to understand which forms of Tau should be targeted in a Tau-based therapeutic approach.
1. Jackson, S. J. *et al.* Short Fibrils Constitute the Major Species of Seed-Competent Tau in the Brains of Mice Transgenic for Human P301S Tau. *J. Neurosci.* **36,** 762-72 (2016). 2. Takeda, S. *et al.* Neuronal uptake and propagation of a rare phosphorylated high-molecular-weight tau derived from Alzheimer's disease brain. *Nat. Commun.* **6,** 8490 (2015). 3. Holmes, B. B. *et al.* Proteopathic tau seeding predicts tauopathy in vivo. *Proc. Natl. Acad. Sci. U. S. A.* **111,** E4376-85 (2014).

Disclosures: **A. Marreiro:** Other; PhD grant partially funded by Janssen Pharmaceutica. **K. Van Kolen:** A. Employment/Salary (full or part-time): Janssen Pharmaceutica. **W. Bruinzeel:** A. Employment/Salary (full or part-time): Janssen Pharmaceutica. **D. Van Dam:** None. **P.P. De Deyn:** None. **L. Temmerman:** None. **G. Depuydt:** None. **L. Schoofs:** None. **M.H. Mercken:** A. Employment/Salary (full or part-time): Janssen Pharmaceutica.

Poster

694. In Vitro and In Vivo Analysis of Tau Pathology

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 694.03/O13

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG017753

Title: Tau association with protein tyrosine phosphatase SHP2: mechanism and cellular location

Authors: Y. KIM[1], M. B. FRANCIS[1], C. J. LEUGERS[1], *G. LEE[2];
[1]Univ. of Iowa, Iowa City, IA; [2]Intrnl. Med., Univ. Iowa, Iowa City, IA

Abstract: The microtubule-associated protein tau is involved in regulating microtubule assembly and function in neurons. Tau function is regulated by phosphorylation and we have previously shown that tau is tyrosine phosphorylated. Here we report that tau co-immunoprecipitated with protein tyrosine phosphatase SHP2 in PC12-derived cell lines as well as in COS7 cells expressing tau by transfection. To investigate the role of phosphorylation in the tau-SHP2 association, we performed *in vitro* binding assays where *E.Coli* synthesized tau was added to SHP2 immunoprecipitated from COS7 cells. The assays confirmed the association, suggesting that phosphorylation of tau was not required for the SHP2 association. However, we also found that phosphomimetic tau (T231D) bound to SHP2 approximately twelve-fold higher than wild type tau, suggesting that phospho-T231 promoted SHP2 association. Using several tau deletion fragments, we found that tau residues 256-282, which contain the microtubule binding repeat 1 of tau, were essential for the tau association with SHP2, suggesting that phosphorylation of tau at Thr231 increased the accessibility of tau residues 256-282 for SHP2 binding. To visualize the spatial localization of the tau-SHP2 complexes in cells, *in situ* proximity ligation assays (PLAs) were conducted using non-neuronal (COS7) or neuronal (PC6-3) cells where tau was expressed by transfection. Tau-SHP2 complexes appeared as a punctate pattern, with 3D reconstruction of the confocal images suggesting that the tau-SHP2 complexes were localized on the plasma membrane. In PC6-3 cells, complexes of endogenous tau and SHP2 were also found on the plasma membrane. When cells were stimulated with EGF (COS7) or NGF (PC6-3), activated SHP2 increased dramatically and complexes of tau and activated SHP2 were detected. Relative to non-stimulated PC6-3 cells, NGF-stimulated cells also exhibited an increase in the number of complexes between tau and total SHP2. The increase in tau-SHP2 complexes was probably, in part, due to the increase in the levels of phospho-Thr231-tau following NGF stimulation, which we had found using both immunoprecipitation and flow cytometry. Lastly, *in vitro* phosphatase assays, performed on tyrosine phosphorylated full-length tau or on tau peptide, showed that SHP2 dephosphorylated phospho-Tyr18- tau, indicating that phospho-Tyr18-tau is a substrate for SHP2. Given the regulation of phospho-Tyr18-tau we have previously reported for Alzheimer's disease brain, SHP2 might be active during neurodegeneration.

Disclosures: Y. Kim: None. **M.B. Francis:** None. **C.J. Leugers:** None. **G. Lee:** None.

Poster

694. In Vitro and In Vivo Analysis of Tau Pathology

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 694.04/O14

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Characterizing the depolarization-dependent synaptic release of tau

Authors: *E. E. MIYOSHI, T. BILOUSOVA, K. H. GYLYS;
UCLA, Los Angeles, CA

Abstract: Alzheimer's disease (AD) is a neurodegenerative disorder characterized by two main neuropathological hallmarks—amyloid beta plaques and neurofibrillary tangles. Neurofibrillary tangles are formed from hyperphosphorylated tau filaments. Tau is a microtubule-associated protein, and its sequestration leads to the disruption of microtubule stability and the formation of highly toxic intermediate tau oligomers, resulting in massive neuronal loss and dementia. It has been proposed recently that tau pathology can synaptically propagate through the brain in an activity-dependent manner. We have used human brain-derived synaptosomes (resealed presynaptic terminals) from AD and control subjects to assess synaptic tau release *in situ*. Our data shows that depolarization of AD synaptosomes using a high-potassium solution induced discharge of a mixture of tau oligomers and monomers. Depolarization of the synaptosomes was confirmed by the anionic fluorescent dye $DiBAC_4(3)$. Western blot analysis using tau proteoform-specific antibodies revealed that the majority of released tau is C-terminally truncated and also part of it is present as SDS stable oligomers. Uptake of the released tau oligomers may induce tau oligomerization in recipient cells, accelerating the propagation of the pathology. Synaptosomes from cryopreserved human brain autopsy samples represent a unique system for investigation of synaptically released tau proteoforms.

Disclosures: **E.E. Miyoshi:** None. **T. Bilousova:** None. **K.H. Gylys:** None.

Poster

694. In Vitro and In Vivo Analysis of Tau Pathology

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 694.05/O15

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NRF-2015R1C1A1A02037282

Title: Identification of tau internal segment for efficient internalization

Authors: *K. CHO, Y. JANG, D. KIM, S. YOON;
Asan Med. Ctr., Seoul, Korea, Republic of

Abstract: Propagation of tau aggregates is critical for tauopathy in Alzheimer's disease. In many progressive neurodegenerative disease, various protein aggregates such as tau, amyloid-β, huntingtin and α-synuclein shows prion-like spreading and infectivity. Recent studies suggest that cellular uptake of Tau is mediated by macropinocytosis. However, internal domain involved in tau internalization is unknown. Here we identified tau domain involved in efficient internalization through in silico prediction of cell permeable peptide site in Tau sequence. Recombinant GFP-tau which has been deleted this segment showed slower cellular uptake than GFP-Tau control. This segment deleted GFP-tau was resistant to treatment of sodium cholate, inhibitor for HSPG critical for tau macropinocytosis, suggesting that identified tau segment is important for tau internalization. Our study demonstrated more precise mechanism for tau internalization and provided novel target for development of anti-tauopathy.

Disclosures: K. Cho: None. **Y. Jang:** None. **D. kim:** None. **S. yoon:** None.

Poster

694. In Vitro and In Vivo Analysis of Tau Pathology

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 694.06/O16

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Roy and Christine Sturgis Charitable Trust

UAMS S.T.O.P. Alzheimer's Fund

Title: Depletion of laforin portends "molecular crowding" in Alzheimer's disease: Implications for protein aggregation and the role of glycemic derangement in neurodegeneration

Authors: K. WOLFE[1], S. Y. AGHDAM[2], S. Z. IMAM[3,2], *S. W. BARGER[2,4];
[1]Col. of Med., [2]Dept Geriatrics, Univ. of Arkansas for Med. Sci., Little Rock, AR; [3]Div. of Neurotoxicology, Natl. Ctr. for Toxicological Res., Jefferson, AR; [4]Geriatric Research, Educ. and Clin. Ctr., Central Arkansas Veterans Healthcare Syst., Little Rock, AR

Abstract: Protein aggregation is a common element of all neurodegenerative diseases, suggesting a particular vulnerability of neurons to this phenomenon. One potential explanation is the unique electrostatic status conferred by the high concentration of RNA in neuronal cytosol, which can be more three orders of magnitude that of other cell types. This elevates the potential for "molecular crowding," a phenomenon by which proteins lose solubility due to neighboring hygroscopic macromolecules that compete for their interactions with polar water molecules. Glycogen is an ideal mediator of molecular crowding, and the high neuronal concentration of RNA—itself a highly charged, hygroscopic molecule—may explain why glycogen levels are typically kept very low in neurons. Neurons constitutively express glycogen synthase, but it is degraded by a ubiquitin-proteasome system requiring an adapter known as laforin. The protein derives its name from Lafora's disease, some cases of which arise from loss-of-function mutations of the laforin gene (*EPM2A*); the resulting neuronal accumulation of glycogen synthase and its product results in seizures and dementia. We have documented a 50% reduction of laforin expression in Alzheimer's disease temporal-lobe cortex. We also find that primary cultured neurons, unlike most cells, reduce their expression of laforin under low-glucose conditions. This may occur as a consequence of decreased levels of the transcription factor hypoxia-inducible factor 1α (HIF1α), which has four potential binding sites in the *EPM2A* promoter and is likewise depleted in Alzheimer's brain. These events may explain the age-related accumulation of polyglucosan aggregates known as corpora amylacea, which have been connected to axonal transport disruptions in Alzheimer's disease. They may also contribute to the documented aggregation of tau in laforin-knockout mice. Implications for hypotheses about the role of peripheral glucose tolerance and insulin dysregulation in Alzheimer's disease will be discussed.

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Poster

694. In Vitro and In Vivo Analysis of Tau Pathology

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 694.07/O17

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Labex DISTALZ

Région Hauts-de- France

CHRU Lille

Université Lille 2

Inserm

FEDER

Title: Tools development for studying a new N-terminally truncated Tau species and its relevance to Alzheimer disease

Authors: C. LEGHAY[1], M. DERISBOURG[1], D. DEMEYER[1], S. EDDARKAOUI[1], R. CAILLIEREZ[1], V. BUÉE-SCHERRER[1], D. BLUM[1], V. DERAMECOURT[2], L. BUÉE[1], *M. HAMDANE[1];
[1]Alzheimer & Tauopathies, UMR 1172 Inserm, Univ. De Lille, CHU Lille, Lille Cedex, France; [2]CMRR, Hôpital Salengro, Univ. Lille, Lille, France

Abstract: Tau protein plays an important role in Alzheimer's Disease (AD) where it is found aggregated and abnormally modified in degenerating neurons. Truncation is among post-translational modifications of Tau and its role in AD pathological process is far from being elucidated. By using a dedicated proteomics approach (liquid chromatography coupled to tandem mass spectrometry), we have identified new N-terminally truncated Tau species (Trunc-Tau) from human brain. Our interesting findings provided us the basis for the development of appropriate immunological tools with which to monitor these new species and to establish their role in AD physiopathological process. We have focused our work on an N-terminally Trunc-Tau species starting at the middle of Tau exon 1 sequence to develop a specific monoclonal antibody. This antibody is a mandatory tool: 1) to uncover the consequences of this N-terminal truncation on Tau function and properties; 2) to establish whether there is an association between this new Trunc-Tau species and AD. We have succeeded in development of an antibody that specifically labels the Trunc-Tau species without any cross reactivity with full-length Tau. We performed immunohistochemistry (IHC) using brains from well characterized AD patients and control subjects (Lille Neurobank). Our preliminary data identified the new Trunc-Tau species in AD neurofibrillary tangles while no immunoreactivity was observed in control brains. We are currently performing IHC using a large number of brain samples to establish whether the new Trunc-Tau species is a signature of AD and other Tauopathies. Besides, we have established inducible stable cell lines expressing the full-length Tau protein and the Trunc-Tau species in order to analyze functional and biochemical properties of the new Trunc-Tau, compared to its related full-length Tau. This work will provide new knowledge on Tau biology and the physiopathological process of AD.

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Poster

694. In Vitro and In Vivo Analysis of Tau Pathology

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 694.08/O18

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Mitchell Center for Neurodegenerative Disease

Cullen Trust

Title: Toxic synergy between amyloid aggregates in disease and their role in tau oligomeric strain formation

Authors: *R. KAYED, J. E. GERSON, D. L. CASTILLO-CARRANZA, U. SENGUPTA, M. J. GUERRERO-MUNOZ;
Neurol., Univ. of Texas Med. Br. Dept. of Neurol., Galveston, TX

Abstract: Recent studies suggest that the toxicity of tau in disease may rely on its prion-like characteristics, including the ability to seed the aggregation of natively unfolded protein and the transport of aggregates from one cell to another. A growing body of research shows that the most toxic and efficient seeds in disease are tau oligomers, rather than insoluble, fibrillar aggregates. Furthermore, evidence suggests that tau aggregates may exhibit conformational diversity important for their toxicity, as seen in prion disorders. However, the relevance of other aggregant proteins to the formation of tau strains has not been well-established though the most prevalent neurodegenerative disorders—Alzheimer's (AD) and Parkinson's disease (PD)—are mixed protein pathology diseases. We have shown that amyloid-â (Aâ) and á-synuclein oligomers colocalize with oligomeric tau in AD, PD and Lewy body dementia brain tissue. Moreover, oligomeric Aâ and á-synuclein can cross-seed the aggregation of tau. Studies show that much of the toxicity of the two proteins is dependent on the presence of tau, highlighting the toxic synergy between tau and other amyloid proteins. Furthermore, we find that targeting oligomeric tau in mouse models over-expressing mutated amyloid precursor protein and á-synuclein protects against behavioral deficits and pathology associated with disease-like phenotypes. Thus, we evaluated tau oligomeric strains derived from mixed pathology diseases and pure tauopathies and found that oligomers could be differentiated based on stability as well as recognition with tau oligomer-specific monoclonal antibody (TOMA) clones. In order to directly investigate secondary amyloidosis and tau oligomeric strain formation and the effect on toxicity, tau was cross-seeded with Aâ and á-synuclein oligomers to form distinct tau oligomeric strains with differing levels of toxicity when applied to neuroblastoma cells in culture. These results highlight the importance of studying the diverse oligomeric structures of complex mixed pathology disorders and suggest that these diseases will likely best be targeted using personalized, combination therapy approaches that target the most toxic strains.

Disclosures: **R. Kayed:** None. **J.E. Gerson:** None. **D.L. Castillo-Carranza:** None. **U. Sengupta:** None. **M.J. Guerrero-Munoz:** None.

Poster

694. In Vitro and In Vivo Analysis of Tau Pathology

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 694.09/P1

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Byrd Small Grant Program

Title: Identifying novel interactions of the Hsp90 kinase co-chaperone Cdc37 *In vivo* and *In vitro*

Authors: ***M. NARAYAN**[1,2], U. K. JINWAL[2];
[1]Pharmaceut. Sci., Univ. of South Florida, Tampa, FL; [2]Pharmaceut. Sci., Byrd Alzheimer's Institute-University of South Florida, Tampa, FL

Abstract: The co-chaperone Cdc37 specifically recruits kinases to the Hsp90 complex for stabilization and folding. The Hsp90-Cdc37 complex is known to regulate kinases involved in the phosphorylation of tau, a microtubule associated protein. Tau hyperphosphorylation and formation of neurofibrillary tangles is a major pathological hallmark of Alzheimer's disease (AD). Our group has previously shown that Cdc37 plays a role in preserving hyperphosphorylated tau. However, the mechanism of how this occurs is poorly understood. While Cdc37 has been shown to interact with numerous kinases, we hypothesized that identification of the components of the Cdc37 interactome in AD brain tissue would lead a better understanding of Cdc37 function in tau biology. For *in vivo* identification of novel Cdc37-binding proteins, five AD patient and five age-matched control brain tissue samples were screened for phosphorylated tau levels by Western blotting. Three samples from each group were chosen based on robust phosphorylated tau levels for immunoprecipitation (IP) using a Cdc37 antibody. IP samples were processed and analyzed by mass spectrometry (MS). Briefly, samples were digested to produce tryptic peptides and desalted peptides were injected into the spectrometer. Data from MS analysis were matched subsequently against a human peptide database. A total of 110 proteins were identified, and upon further data analysis we found 39 novel proteins that were found to interact with Cdc37 in AD cases and 7 proteins that were found only in controls. Several of these proteins are implicated in signaling via the PI3K / Akt, NF-B, JNK and TGFβ pathways. Novel interactors of Cdc37 included Shroom3, β-catenin and annexin A2. To validate these interactions we used an *in vitro* cell culture model and found that Cdc37 does interact with these proteins. Overall, data from these *in vivo* and *in vitro* studies not only

help in better understanding of the functional role of Cdc37, but also reveal novel Cdc37-interacting proteins and pathways in AD that might lead to the identification of new AD drug targets.

Disclosures: **M. Narayan:** None. **U.K. JInwal:** None.

Poster

694. In Vitro and In Vivo Analysis of Tau Pathology

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 694.10/P2

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: HHMI SOMAS-URM 52006292

Title: Sod2 rescues p301l tau-transfected primary neurons

Authors: ***K. A. BASKERVILLE**, N. M. CHIKWEM, N. WEIGEL;
Biol., Lincoln Univ., Lincoln University, PA

Abstract: Alzheimer’s disease is characterized by two hallmark pathologies – neurofibrillary tangles and beta amyloid plaques. Oxidative stress plays an apparent role in the pathogenesis of Alzheimer’s disease. Neurofibrillary tangles result from the filamentous aggregation of hyperphosphorylated tau, leading to neurodegeneration. After the hyperphosphorylation of tau, we suspect that antioxidant systems within neurons become progressively dysfunctional and, thus, are unable to scavenge reactive oxygen species effectively. The purpose of this study was to investigate whether mitochondrial superoxide dismutase (SOD2), an antioxidant, can rescue hyperphosphorylated tau-transfected neurons (cortical and hippocampal) in primary cell culture from neurodegeneration and oxidative damage. Primary cortical and hippocampal neurons were co-transfected with a common form of hyperphosphorylated tau, P301L, construct and a SOD2 construct; GFP DNA constructs and vector constructs served as controls. P301L-transfection alone induced degeneration and oxidative stress in primary cultured neurons but were rescued by SOD2. “Sick,” i.e. degenerated, neurons displayed morphological changes in their neurites and cell bodies. Oxidative damage (lipid peroxidation and nucleic acid damage) was observed in the P301L-transfected neurons. The number of healthy, intact neurons increased after mutant tau and SOD2 co-transfection; 70% of neurons co-transfected with P301L and SOD2 compared to 45% healthy with P301L tau transfection alone. We suspect that SOD2 protects the P301L tau-transfected neurons from oxidative stress, thereby reducing degeneration.

Disclosures: **K.A. Baskerville:** None. **N.M. Chikwem:** None. **N. Weigel:** None.

Poster

694. In Vitro and In Vivo Analysis of Tau Pathology

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 694.11/P3

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Else Kröner-Fresenius-Stiftung

Title: Disassembly of the MID1-PP2A complex causes dephosphorylation of Tau

Authors: *F. MATTHES[1], S. SCHWEIGER[3], M. M. HETTICH[2], D. EHNINGER[2], S. KRAUSS[1];
[1]AG Krauss, [2]AG Ehninger, DZNE, Bonn, Germany; [3]Inst. for Human Genet., Univ. of Mainz, Mainz, Germany

Abstract: Alzheimer's disease (AD) is the most common form of dementia and the most prominent neurodegenerative disorder associated with aging. One of the pathological hallmarks observed in AD patients' brains is the development of paired helical filaments (PHFs), which are composed of hyperphosphorylated Tau protein dissociating from microtubules. The most important phosphatase that is capable of dephosphorylating Tau at AD-specific phosphorylation sites is protein phosphatase 2A (PP2A).
We show that disassembly of the MID1-PP2A complex by a natural polyphenol induces PP2A activity and significantly reduces Tau phosphorylation at PP2A-dependent epitopes in cultured primary neurons. The MID1 protein is a ubiquitin ligase that mediates ubiquitin-specific modification and degradation of the catalytic subunit of PP2A, specifically at the microtubules. Western blot and quantitative real-time PCR analyses indicate that disassembly of the MID1-PP2A complex leads to reduced MID1 mRNA and protein levels, suggesting a self-regulating effect of MID1 on its own mRNA. In line with our observations *in vitro*, treatment of mice with a MID1-PP2A destabilizing compound resulted in dephosphorylation of Tau *in vivo*.
Our data suggest a promising role for drugs interfering with the MID1-PP2A complex as a novel approach for reducing Tau phosphorylation in the prophylaxis and therapy of AD and other tauopathies.

Disclosures: F. Matthes: None. **S. Schweiger:** None. **M.M. Hettich:** None. **D. Ehninger:** None. **S. Krauss:** None.

Poster

694. In Vitro and In Vivo Analysis of Tau Pathology

Location: Halls B-H

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Program#/Poster#: 694.12/P4

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: DST-SERB EMR/2014/000336

Centre for Biomedical Engineering, IIT Gandhinagar

Title: Charge interplay in tau fibrillization: a mechanistic insight

Authors: *G. VISWANATHAN[1], S. GUPTA[2];
[1]Biol. Engin., Indian Inst. of Technol. Gandhinagar, Gandhinagar, India; [2]Biol. Engin., Indian Inst. of Technology, Gandhinagar, Gandhinagar, India

Abstract: Tau is an intrinsically disordered protein responsible for maintaining the stability of axonal microtubules in neurons. In AD, abnormal Post Translational Modifications (PTMs) may cause detachment of Tau which undergoes complex aggregation pathways to form Neurofibrillary Tangles (NFTs). Traditionally, heparin induced tau aggregation referred as pseudophosphorylation is used as a model to understand the aggregation mechanism. Since heparin is a polyanion, it attracts unmodified positively charged tau and promotes aggregation in a shorter time frame. But *in vivo*, tau undergoes several PTMs at the same temporal scale which may be charge driven (e.g. phosphorylation) or uncharged (e.g. glycation). We asked whether these modified tau species aggregates in the same manner *in-vitro*? Using Thioflavin T fluorescence based aggregation assay we mapped heparin induced unmodified tau aggregation in real-time but when we used chemically phosphorylated tau, the trajectories diverged indicating a change in pathway as well. Addition of negative charge on tau tends to repel heparin causing aggregation to be much slower. We verified this hypothesis by using different ratios of modified to unmodified tau. On the other hand, these modified tau species have a unique tendency to aggregate independently without any aggregation inducer. Atomic Force Microscopy (AFM) images of self-aggregating chemically modified tau resemble that of PHFs found in human brain. This charge interplay between phosphorylated tau and heparin can be very crucial in understanding the intricate pathways of tau aggregation. We observed that heparin based *in-vitro* tau aggregation models are not sufficiently reliable for high throughput screening of small molecule and peptide based inhibitors. Moreover, to mimic tau fibrillization, we require ~1mg of tau protein per well as tau aggregates at higher concentration *in-vitro*. To increase the availability of tau, we have further developed a strategy of co-transformation where we supply deficient tRNAs to *E. coli* which codes for rare amino acids to increases the rate of translation. We have also developed a heat based quick and clean tau extraction method which can provide assay-ready tau from cell pellet in less than an hour.

Disclosures: **G. Viswanathan:** None. **S. Gupta:** None.

Poster

694. In Vitro and In Vivo Analysis of Tau Pathology

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Mitchell Center for Neurodegenerative diseases

Sealy Center for Vaccine Development

Cullen Trust

Title: Tau oligomers and the dysregulated proteasome in Alzheimer's disease

Authors: ***A. N. NILSON**, J. DEGER, J. GERSON, R. KAYED;
Mitchell Ctr. for Neurodegenerative diseases, Neuroscience, Neurol., Univ. of Texas Med. Br., Galveston, TX

Abstract: Tau is a microtubule-associated protein that pathologically aggregates into inclusions and neurofibrillary tangles in Alzheimer's disease (AD) and other neurodegenerative diseases. However, there is evidence that synapse loss and cell death occur prior to, or independently of, the formation of neurofibrillary tangles. Smaller, soluble aggregates of tau, known as oligomers, appear early in disease and have been shown to be the primary toxic entity in disease. In addition, it is known that tau oligomers can be modified by both mono- and poly-ubiquitination, which may play a role in the dysregulation of the ubiquitin-proteasome system in neurodegeneration. Poly-ubiquitination typically targets a protein for degradation. The role of small ubiquitin-like modifiers (SUMO) on tau oligomer formation, stability, and toxicity is less understood. Thus, we investigated the role of ubiquitination versus sumoylation of tau oligomers in vitro and in post-mortem brain samples, as well as how proteasome inhibition influences tau oligomer toxicity. Using immunofluorescence and biochemical analyses with tau oligomer-specific antibodies, we systematically dissected the relationship between tau oligomers, ubiquitin, and SUMO. We found that tau oligomers were preferentially ubiquitinated in AD while they were sumoylated in controls. Moreover, inhibition of the proteasome increases the presence of hyperphosphorylated tau as well as other aggregation prone proteins. These results suggest that the pattern of post-translational modification in conjunction with the proteasome is altered in AD, exacerbating the toxicity of tau oligomers and leading to their accumulation and the buildup of other aggregation prone proteins. This may lead to a cycle in which tau oligomers

inhibit the proteasome, increasing the abundance of oligomeric tau, thereby maintaining proteasome inhibition, all of which increases tau toxicity. Thus, increasing proteasome activity or preventing the interaction of tau oligomers with the proteasome may slow the progression of AD.

Disclosures: **A.N. Nilson:** None. **J. Deger:** None. **J. Gerson:** None. **R. Kayed:** None.

Poster

694. In Vitro and In Vivo Analysis of Tau Pathology

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 694.14/P6

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH

MassGeneral Hospital

Title: Liquid-liquid phase separation of human tau protein

Authors: ***S. WEGMANN**[1], B. EFTEKHARZADEH[1], A. M. PALUD[2], J. P. TAYLOR[2,3], B. T. HYMAN[1];
[1]Neurol., Mass Gen. Hosp. / Harvard Med. Sch., Charlestown, MA; [2]Dept. of Cell and Mol. Biol., St. Jude Children's Res. Hosp., Memphis, TN; [3]Howard Hughes Med. Inst., Chevy Chase, MD

Abstract: Liquid-Liquid Phase Separation (LLPS) is a major principle underlying cellular organization. Because LLPS is present even in prokaryotes, this organizational strategy likely evolutionarily preceded the elaboration of intracellular membrane-derived organelles; it is for example used to locally increase the concentration of certain proteins and RNA molecules, independently of cellular organelles. The formation of liquid droplets in the cell is facilitated by intrinsically unstructured multivalency domains with low folding propensity (low-complexity domains) in the contributing proteins and drives, for example, the spatially asymmetry of P-bodies in germ cells, the rapid and reversible assembly of RNP granules, but also the assembly of microtubules during spindle formation, membrane receptor clustering, orchestration of DNA repair, the assembly of transcription factories, and control of permeability through the nuclear pore. Tau, alike other microtubule binding proteins, contains a long unstructured N-terminus, which projects into the cytoplasm when bound to microtubules (projection domain) and has biochemical properties similar to low complexity domains reported in LLPS performing proteins. Here we tested if the intrinsically unfolded protein tau - or its fragments - can form liquid

droplets in a cellular environment. We used microscopy and different biophysical techniques to examine LLPS characteristics of recombinant human tau and its different fragments (wild-type, mutant P301L, full-length (tau441), repeat domain, N-terminus (tau256)). We then tested the cellular function of tau256 LLPS in cells and primary neurons. The N-terminal half of human tau (tau256) undergoes LLPS when exposed to a molecular crowded environment. At physiological concentrations of tau (5-10 uM), tau256 forms viscous droplets in a ion strength dependent manner; this phase transition appears independent of temperature, and tau constructs comprising the repeat domain (microtubule binding domain) cannot undergo LLPS. Accordingly, expression of GFP-tau256 but not GFP-tau441 leads to intracellular droplets in cells and in neurons. The unstructured N-terminus of tau has biophysical properties enabling LLPS in a cellular environment. Intracellular droplets of tau256 might occur after cleavage of tau by proteases such as calpain or thrombin, which releases the N-terminus of tau into the cytosol. The biological function of tau droplet formation is unknown, but by analogy to other systems could represent a combined phase separation with RNPs such as hnRNPA1, TDP-43 and FUS, or a microtubule-associated function of the N-terminal projection domain.

Disclosures: **S. Wegmann:** None. **B. Eftekharzadeh:** None. **A.M. Palud:** None. **J.P. Taylor:** None. **B.T. Hyman:** None.

Poster

695. Biochemical Manipulations in Animals: Prevention of Alzheimer's Disease

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 695.01/P7

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH R00 AG037716

Title: Transient cerebral ischemia promoters brain mitochondrial dysfunction and exacerbates cognitive impairments in young 5xFAD mice

Authors: ***J. TIAN**[1], L. LU[2], E. GAUBA[3], L. GUO[3], H. DU[3];
[1]Mol. and Cell biology Dept., The Univ. of Texas At Dallas, Richardson, TX; [2]Dept. of Neurology, Shandong Provincial Hosp. Affiliated to Shandong Univ., Ji Nan, China; [3]The Univ. of Texas at Dallas, Richardson, TX

Abstract: Alzheimer's disease (AD) is heterogeneous and multifactorial neurological disorder; and the risk factors of AD still remain elusive. Recent studies have highlighted the role of vascular factors in promoting the progression of AD and have suggested that ischemic events increase the incidence of AD. However, the detailed mechanisms linking ischemic insult to the

progression of AD is still largely undetermined. In this study, we have established a transient cerebral ischemia model on young 5xFAD mice and their non-transgenic (nonTg) littermates by the transient occlusion of bilateral common carotid arteries. We have found that transient cerebral ischemia significantly exacerbates brain mitochondrial dysfunction including mitochondrial respiration deficits, oxidative stress as well as suppressed levels of mitochondrial fusion proteins including optic atrophy 1 (OPA1) and mitofusin 2 (MFN2) in young 5xFAD mice resulting in aggravated spatial learning and memory. Intriguingly, transient cerebral ischemia did not induce elevation in the levels of cortical or mitochondrial Amyloid beta (Aβ)1-40 or 1–42 levels in 5xFAD mice. Therefore, the simplest interpretation of our results is that young 5xFAD mice with pre-existing AD-like mitochondrial dysfunction are more susceptible to the effects of transient cerebral ischemia; and ischemic events may exacerbate dementia and worsen the outcome of AD patients by exacerbating mitochondrial dysfunction.

Disclosures: J. Tian: None. **L. Lu:** None. **E. Gauba:** None. **L. Guo:** None. **H. Du:** None.

Poster

695. Biochemical Manipulations in Animals: Prevention of Alzheimer's Disease

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 695.02/P8

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Enzymatic digestion of extracellular matrix modulates cortical microglia response to damage in the 5xFAD mice.

Authors: *S. STOYANOV[1], W. SUN[1], I. CHOI[1], K. REYMANN[1], A. ISAEVA[2], M. RIEK-BURCHARDT[3], J. NEUMANN[4], A. DITYATEV[1];
[1]DZNE E.V., Magdeburg, Germany; [2]Dept. of Med. Chem. and Biochem., Med. Univ., Sofia, Bulgaria; [3]Institue for molecular and clinical immunology,, Magdeburg, Germany; [4]Dept. of Neurol., Otto-von-Guericke Univ., Magdeburg, Germany

Abstract: Pathological deposition of amyloid-beta (A-beta) is one of the major hallmarks of Alzheimer’s dementia (AD). The incidence of AD and other neurodegenerative diseases is rising with age, and thus often contributing to common comorbidity state of mixed dementia. Microglia are the primary immune effector cells of the brain. In resting state their highly motile processes continually survey the brain parenchyma and transiently contact synaptic elements (Tremblay et al., 2010). In neuropathological conditions microglia cells rapidly become activated, thicken and retract their processes, migrate to the site of inflammation, and proliferate. In addition, they also contribute to the presentation of antigens, phagocytosis of cellular debris, and secretion of proteases that promote cell motility, myelin and extracellular matrix degradation (Avignone et

al., 2015). However, little is known about the role of extracellular matrix (ECM) molecules in the regulation of microglia function. Thus, we aimed to study the effects of ECM removal by chondroitinase ABC (ChABC) on microglia response to a minor brain lesion (laser ablation of a single cell) in the 5xFAD mouse model of AD. *In vivo* multiphoton laser microscopy imaging was used to study microglial behavior in the retrosplenial cortex (RSC) of 3-4 months old CX3CR-1-eGFP mice cross-bred with 5xFAD mice. Microglial morphology and dynamics were studied before and after single microglia cell laser ablation (SMCLA) upon injection of ChABC or vehicle for 24, 48 and 72h. A-beta plaques were traced by intravenous injection of the *in vivo* marker Methoxy-XO4. Microglia cells displayed activated phenotype in RSC of 5xFAD/CX3CR-1-eGFP mice, but not in WT/CX3CR-1-eGFP mice. Interestingly, at the age studied, amyloid plaques were not yet visible after injection of Methoxy-X04. Measurement of basal process-motility revealed a significant impairment in single microglia cell of 5xFADxCX3CR-1-eGFP mice when compared to controls. Moreover, kinetic deficits of total processes spreading were observed in 5xFAD-xCX3CR-1-eGFP microglia at the population level in response to SMCLA. Intracerebral injection of ChABC improved microglia dynamics comparable to WT controls in 5xFADxCX3CR-1-eGFP mice. Furthermore, we detected significantly reduced recruitment of microglial cells to the site of damage in 5xFADxCX3CR-1-eGFP mice 24h after laser ablation compared to the CX3CR-1-eGFP group. ChABC ameliorated these defects 48h upon application. Our findings suggest that microglia may lose adequate response to inflammatory stimulus at early stages of AD, but this response could be restored after administration of ChABC.

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Poster

695. Biochemical Manipulations in Animals: Prevention of Alzheimer's Disease

Location: Halls B-H

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: All authors are employees of AbbVie. The design, study conduct, and financial support for this research were provided by AbbVie. AbbVie participated in the interpretation of data, review, and approval of the publication.

Title: Raising *In vivo* microdialysis to the next level: Evaluation of long-term extracellular amyloid-β and tau in mouse models of Alzheimer’s disease

Authors: *M. MEINHARDT, M. KATSUR, G. PLOTZKY, E. CHATZIKONSTANTINOU, S. BENOE, I. MAIRHOFER, J. HOPPE, S. TALMON, K. BUCK;
Neurosci. Res., Abbvie Deutschland Gmbh & Co. KG, Ludwigshafen, Germany

Abstract: Amyloid-β and tau are key players in Alzheimer's disease (AD), both of which have been found as pathological species in the extracellular space, suggesting a significant role of amyloid-β and tau in this compartment during AD progression. The only possible approach to monitor these proteins in the extracellular space *in vivo* is cerebral microdialysis. This technique is classically used to measure small molecules but with several modifications also allows protein sampling. These adaptations add another level of complexity to an already sophisticated technique. Therefore, particular attention should be paid to various experimental factors to achieve high quality results and enhance our understanding of the role of extracellular amyloid-β and tau in AD. We here tackled these challenges by setting up a push/pull microdialysis technique, equipped with a 1MDa molecular weight cut-off membrane. Due to the fact that molecular dynamics of proteins require long-term monitoring, we optimized the set-up allowing continuous sampling for up to 72h. Experimental parameters such as the flow rate (0.6 vs. 1µL/min), the type/material of the membrane, additives to the perfusion media (Dextran vs. 0.2/4% BSA) as well as the impact of coating procedures for the system turned out to be very critical in achieving long-term stability. Moreover, we used miniaturized microdialysis probes allowing to place multiple probes in mice as well as an optimized analytical readout enabling simultaneous quantification of amyloid-β and tau within the same sample of wild-type and transgenic mice. Finally, we set the focus on investigating the disruption of the blood-brain-barrier, which inevitably occurs with probe insertion, and is particularly important for PK/PD studies. In conclusion, this in-depth characterization of an amyloid-β and tau microdialysis method increases experimental possibilities and will further enhance our understanding of these proteins in the extracellular compartment. Therefore, this set-up provides a powerful tool for uncovering their role in the disease progression and facilitates experiments investigating the efficacy of treatments and the search for new biomarkers.

Disclosures: **M. Meinhardt:** A. Employment/Salary (full or part-time): AbbVie Deutschland GmbH & Co. KG. **M. Katsur:** A. Employment/Salary (full or part-time): AbbVie Deutschland GmbH & Co. KG. **G. Plotzky:** A. Employment/Salary (full or part-time): AbbVie Deutschland GmbH & Co. KG. **E. Chatzikonstantinou:** A. Employment/Salary (full or part-time): AbbVie Deutschland GmbH & Co. KG. **S. Benoe:** A. Employment/Salary (full or part-time): AbbVie Deutschland GmbH & Co. KG. **I. Mairhofer:** A. Employment/Salary (full or part-time): AbbVie Deutschland GmbH & Co. KG. **J. Hoppe:** A. Employment/Salary (full or part-time): AbbVie Deutschland GmbH & Co. KG. **S. Talmon:** A. Employment/Salary (full or part-time): AbbVie Deutschland GmbH & Co. KG. **K. Buck:** A. Employment/Salary (full or part-time): AbbVie Deutschland GmbH & Co. KG.

Poster

695. Biochemical Manipulations in Animals: Prevention of Alzheimer's Disease

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 695.04/P10

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: RC-IRMS-15-16-02, Hong Kong Baptist University

Title: *In situ* harvest of neural stem cells from subventricular zone and cell replacement in live rat brains

Authors: *Z. S. QING[1], L. KAI LI[2], L. N. P.[2], Y. K. L.[1];
[2]Biol., [1]Hong Kong Baptist Univ., Hong Kong, Hong Kong

Abstract: We have invented a novel method in harvesting neural stem cells (NSCs) from the subventricular zone of rats (Lui et al., *Angew Chemie Int Ed* 52: 12298-12302, 2013). Neural stem cells were harvested from the subventricular zone using *in situ* harvesting procedures. Then use the antibodies to conjugate with the magnetic NPs to enhance the specificity for the stem cell extraction. The antibody-conjugated magnetic nanoparticles (Ab-MNPs) can target and attach to NSCs. The NSCs pellet can be harvest by syringe and then culture in the neuro base medium. The Ab-MNPs shows a low cytotoxicity and will sooner be metabolized during the cell differentiation process. Using this method, neural stem cell can be harvested from live subjects and can be further differentiated into neuronal phenotypes for cell replacement in the brain. Neural stem cells were harvested from the subventricular zone using *in situ* harvesting procedures. Neural stem cells were cultured *in vitro* for 2-3 weeks using a novel nanomatrix. Cell samples were lysed and were assayed for expression of nestin, Tuj-1 and MAP-2 throughout the period. After confirmation of stable expression of Tuj-1 and MAP-2 proteins, living cells were transplanted into the cerebral cortex of the same rat subject for assessment of cell survival. Neuronal cells were found to surviving in the cortex of the animals. Neurites were found to extend from the transplanted neuronal cells and were found to be in close apposition to native neurons. There was no sign of neurological deficits in the animals. In some animals, the neural stem cells were found to be regenerated after 3-4 months after the harvesting procedures. The present results indicate that our method can harvest neural stem cells from live animal subjects. Neural stem cells harvested can be differentiated into neuronal phenotypes and can be used as cell replacement therapy for neurological diseases in the same animal subjects. These results have a strong implication in development into personalized neuronal cell replacement therapy for patients with neurodegeneration.
Key words: Neural stem cells; Nanoparticles; Neurodegeneration
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Disclosures: **Z.S. Qing:** None. **L. Kai Li:** None. **L. N. p.:** None. **Y. K. l.:** None.

Poster

695. Biochemical Manipulations in Animals: Prevention of Alzheimer's Disease

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NMRC/CBRG/0041/2013

Title: Proteasomal Inhibition restores long term potentiation in hippocampal CA1 pyramidal neurons of APP/PS1 mice

Authors: *K. MUTHUKUMARAPPAN, S. SAJIKUMAR;
Natl. Univ. of Singapore, Singapore, Singapore

Abstract: Synthesis of new plasticity proteins and degradation of worn out proteins are required for the maintenance of long term potentiation (LTP), the cellular correlate of memory. The ubiquitin proteasome system (UPS) is the critical protein quality control system regulating this equilibrium during LTP. Blockade of the UPS has been shown to accumulate new plasticity proteins involved in the early induction phase of late LTP. In Alzheimers disease (AD), the induction phase of late LTP is particularly impaired. In this study we have shown that LTP impairment can be restored in APP/PS1 mice by inhibiting the UPS by proteasome inhibitors such as Lactacystin and MG 132.

Disclosures: K. Muthukumarappan: None. **S. Sajikumar:** None.

Poster

695. Biochemical Manipulations in Animals: Prevention of Alzheimer's Disease

Location: Halls B-H

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: MOST-102-2628-B-010-001-MY3

MOST-102-2628-B-010-040-MY3

MOST-103-2628-B-010-040-MY3

MOST-104-2320-B-010-041-MY3

Title: Role of transient receptor potential ankyrin 1 channels in Alzheimer's disease

Authors: *H.-T. LEE;
No 155, Section 2, Li-Nong Street, Natl. Yang-Ming Univ., Taipei, Taiwan

Abstract: Transient receptor potential ankyrin 1 (TRPA1) channel plays an important role in pain and inflammation. However, little is known about the significance of the TRPA1 channel in the pathophysiology of Alzheimer's disease (AD). Wild-type (WT), TRPA1$^{-/-}$, amyloid precursor protein (APP)/presenilin 1 (PS1) transgenic (APP/PS1 Tg) mice,the mouse model of AD, and APP/PS1 Tg/TRPA1$^{-/-}$ mice were used to examine the role of TRPA1 in pathogenesis of AD. Western blot was used for protein expression; immunohistochemistry was used for histological examination. The mouse behaviors were evaluated by locomotion, nesting building, Y-maze and Morris water maze tests; levels of interleukin (IL)-1β, IL-4, IL-6 and IL-10 and the activities of protein phosphatase 2B (PP2B), NF-κB and nuclear factor of activated T cells (NFAT) were measured by conventional assay kits; Fluo-8 NW calcium (Ca^{2+}) assay kit was used for the measurement of intracellular Ca^{2+} level in primary astrocytes and HEK293 cells. The protein expression of TRPA1 channels was higher in brains, mainly astrocytes of the hippocampus, from APP/PS1 Tg mice than WT mice. ablation of TRPA1-channel function in APP/PS1 Tg mice alleviated behavioral dysfunction, Aβ plaque deposition and pro-inflammatory cytokine production but increased astrogliosis in brain lesions. TRPA1 channels were activated and Ca2+ influx was elicited in both astrocytes and TRPA1-transfected HEK293 cells treated with fibrilized $Aβ_{1-42}$; these were abrogated by pharmacological inhibition of TRPA1 channel activity, disruption of TRPA1 channel function or removal of extracellular Ca2+. Inhibition of TRPA1 channel activity exacerbated $Aβ_{1-42}$-induced astrogliosis but inhibited $Aβ_{1-42}$-increased PP2B activation, the production of pro-inflammatory cytokines and activities of transcriptional factors NF-κB and NFAT in astrocytes and in APP/PS1 Tg mice. Pharmacological inhibition of PP2B activity diminished the fibrilized $Aβ_{1-42}$-induced production of pro-inflammatory cytokines, activation of NF-κB and NFAT and astrogliosis in astrocytes. TRPA1− Ca^{2+} − PP2B signaling may play a crucial role in regulating astrocyte-derived inflammation and pathogenesis of AD.

Disclosures: H. Lee: None.

Poster

695. Biochemical Manipulations in Animals: Prevention of Alzheimer's Disease

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 695.07/Q1

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Proteome changes in the cerebrospinal fluid of mouse models for α-amyloidosis and synucleinopathy

Authors: *T. ENINGER[1,3,4], S. A. MÜLLER[2], M. BACIOGLU[1,3,4], M. SCHWEIGHAUSER[3,4], P. J. KAHLE[1,3], S. F. LICHTENTHALER[2,5,6,7], M. JUCKER[1,3], S. A. KAESER[1,3]; [1]German Ctr. for Neurodegenerative Dis., Tuebingen, Germany; [2]Dept. of Neuroproteomics, German Ctr. for Neurodegenerative Dis., Munich, Germany; [3]Dept. of Cell. Neurol., Hertie Inst. for Clin. Brain Res., Tuebingen, Germany; [4]Grad. Training Ctr. of Neurosci., Tuebingen, Germany; [5]Neuroproteomics, Klinikum rechts der Isar, Technische Univ. München, Munich, Germany; [6]Inst. for Advanced Study, Technische Univ. München, Garching, Germany; [7]Munich Ctr. for Systems Neurol. (SyNergy), Munich, Germany

Abstract: Neurodegenerative diseases, such as Alzheimer's (AD) and Parkinson's disease (PD), are of growing importance in the aging population necessitating biomarkers for early diagnosis and treatment response. Due to its close contact to the brain, cerebrospinal fluid (CSF) is a promising source for the discovery of such markers reflecting protein alterations within the central nervous system. Previous work has shown that amyloid precursor protein (APP) transgenic mice mimic alterations of amyloid-β (Aβ) and tau levels in CSF observed in patients with sporadic and familial forms of AD making them a valuable model for translational biomarker research. In the present study we analyzed CSF-proteome changes in two well-established transgenic mouse models for β-amyloidosis and synucleinopathy, the pathological hallmark lesions of AD and PD, respectively. Therefore, we used mice either overexpressing mutant human Presenilin-1 and amyloid precursor protein (APPPS1), or mutated human α-Synuclein (A30P-aSyn) for CSF collection. We applied mass spectrometry-based label-free quantification to assess the CSF proteome in young vs. aged mice of both lines compared to age-matched non-transgenic controls. 1039 and 1269 CSF proteins could be quantified in the APPPS1 and in A30P-aSyn cohorts, respectively. Interestingly, this unbiased shotgun approach did not only reveal age- and transgene-related changes in analytes that have already been associated to AD or PD, like soluble APP, soluble TREM2, ApoE or NfL, but also yielded novel proteins that might be interesting candidates for further evaluation and validation. This dataset provides new insights into CSF protein alterations in models of β-amyloidosis and synucleinopathies and may contribute guidance for the identification of novel biomarkers for human disease.

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Poster

695. Biochemical Manipulations in Animals: Prevention of Alzheimer's Disease

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Program#/Poster#: 695.08/Q2

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: The p3-Alcβ peptide shows an effect to protect neurons from Aβ toxicity.

Authors: *A. UCHIYAMA, A. KIMURA, C. OMORI, S. HATA, T. SUZUKI;
Hokkaido Univ., Sapporo-Shi, Japan

Abstract: Alcadeins (Alcs; Alcα , Alcβ, and Alcγ) are evolutionarily conserved type I transmembrane proteins that undergo the proteolytic cleavages as does amyloid β-protein precursor (APP) (1, 2). Alcs are primarily cleaved by APP α-secretase (ADAM10/17) to produce the N-terminal extracellular ectodomain (sAlc) and the membrane-associated C-terminal fragment (Alc CTF). The Alc CTF is subsequently cleaved by γ-secretase to generate the N-terminal p3-Alc peptide and the C-terminal intracellular cytoplasmic domain (Alc ICD) fragment. Thus, Alcβ secrets major molecular species p3-Alcβ37 into CSF (3), and we found that the level of p3-Alcβ37 significantly decreased in Alzheimer's disease patients. Further study indicates that p3-Alcβ37 suppresses neuronal impairment induced by Aβ42 oligomer in vitro, and we identified the core amino acid sequence composed of 11 amino acids within p3-Alcβ37. Our results suggest that this small peptide composed of 11 amino acids (p3-Alcβ-11) might be a seed or a lead to develop a novel therapeutic agent to Alzheimer's disease. To understand the molecular function of p3-Alcβ to neurons, we explored the target molecule of p3-Alcβ37 and p3-Alcβ-11 peptide. Using the biotinylated p3-Alcβ-11 peptide probe, we isolated candidate neuronal proteins, and the proteins were analyzed with LC-MS. Identification of target protein(s) of p3-Alcβ37 will also contribute for understanding of the physiological function of this endogenously generated peptide.
1) Araki, Y. *et al*. [2003] *J. Biol. Chem.* 278, 49448-49458.
2) Araki, Y. *et al.* [2004] *J. Biol. Chem.* 279, 24343-24354.
3) Hata, S. *et al*. [2009] *J. Biol. Chem.* 284, 36024-36033.

Disclosures: **A. Uchiyama:** None. **A. Kimura:** None. **C. Omori:** None. **S. Hata:** None. **T. Suzuki:** None.

Poster

695. Biochemical Manipulations in Animals: Prevention of Alzheimer's Disease

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 695.09/Q3

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: France-Alzheimer Association

Foundation Plan Alzheimer

Title: Functional and morphological alterations in primates following Alzheimer brain inoculation

Authors: *J. E. KOCH[1], C. GARY[2], F. PETIT[3], Z. HANSS[3], S. J. SAWIAK[4], A.-S. HERARD[3], J.-P. DESLYS[5], E. E. COMOY[5], J.-L. PICQ[3], F. PIFFERI[6], M. DHENAIN[3]; [1]Univ. WI Oshkosh, Oshkosh, WI; [2]Ctr. Natl. de la Recherche Scientifique (CNRS), Fontenay-aux-Roses, France, France; [3]Ctr. Natl. de la Recherche Scientifique (CNRS), Fontenay-aux-Roses, France; [4]Wolfson Brain Imaging Centre, Univ. of Cambridge, Cambridge, United Kingdom; [5]Lab. de physiopathogénie et de prévention des prions et des pathogènes atypiques (L4PA), Fontenay aux Roses, France; [6]UMR7179 CNRS-MNHN, BioAdapt,, Brunoy, France

Abstract: Alzheimer disease (AD) is characterized by the accumulation of misfolded beta-amyloid (AB) and tau proteins. Acceleration of AD-related protein aggregation following human AD brain homogenates inoculation has been demonstrated in transgenic rodents. Studies in primates are however not as conclusive. This study's goal was to determine the impacts of experimental transmission on both brain function and integrity in mouse lemur primates. Twelve adult mouse lemurs, (3 to 4 yrs.) were bilaterally inoculated in the hippocampus and subjacent cortex with human brain homogenates from AD patients or control (CTRL) age-matched subjects. Longitudinal tests of cognition, EEG and morphology (via MRI) were done up to 18 months post-inoculation followed by of brain tissue immunohistopathology. AD-inoculated animals progressively developed cognitive impairments affecting first long-term memory and later learning abilities. EEG profiles of AD-inoculated lemurs shifted progressively towards fast frequencies compared to CTRL-inoculated lemurs. Compared to the CTRL-inoculated group, AD animals displayed an atrophy of the retrosplenial and posterior cingulate cortices as well as adjacent regions of the limbic connectome Immunohistochemistry revealed few cortical amyloid plaques and sparse cortical amyloid angiopathy in some AD-inoculated animals. We demonstrate for the first time that inoculation with AD brain homogenates induces functional and morphological alterations in primates without induction of immunohistopathological-detectable forms of AB or tau proteins.

Disclosures: **J.E. Koch:** None. **C. Gary:** None. **F. Petit:** None. **Z. Hanss:** None. **S.J. Sawiak:** None. **A. Herard:** None. **J. Deslys:** None. **E.E. Comoy:** None. **J. Picq:** None. **F. Pifferi:** None. **M. Dhenain:** None.

Poster

695. Biochemical Manipulations in Animals: Prevention of Alzheimer's Disease

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 695.10/Q4

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Novel Osmotin inhibits SREBP2 via AdipoR1/AMPK/SIRT1 pathway to improve Alzheimer's disease Neuropathological Deficits

Authors: ***M.-O. KIM**[1], S. ALI SHAH[2], G. YOON[2], M. KIM[2], M. JO[2], M. CHO[2];
[1]Col. of Nature Sci., Dept. of Biol., Gyeongsang Natl. Univ., Gajwa 900, Jinju, Korea, Republic of; [2]Dept. of Biology, Gyeongsang Natl. Univ., Jinju, Korea, Republic of

Abstract: Extensive evidence has indicated that a high rate of cholesterol biogenesis and abnormal neuronal energy metabolism play key roles in Alzheimer's disease (AD) pathogenesis. Here we used osmotin for the first time, a plant protein homolog of mammalian adiponectin, to know its therapeutic efficacy in different AD models. Our results reveal that osmotin treatment modulated adiponectin receptor 1 (AdipoR1) and significantly induced AMPK/SIRT1 activation, reduced SREBP2 expression both *in vitro* and *in vivo* AD models and in Adipo$^{-/-}$ mice. Osmotin via AdipoR1/AMPK/SIRT1/SREBP2 signaling pathway significantly diminished the amyloidogenic Aβ production, abundance and aggregation accompanied by improved pre-and post-synaptic dysfunction, cognitive impairment, memory deficits and most importantly reversed the suppressed LTP in AD mice. Interestingly, AdipoR1, AMPK and SIRT1 silencing not only abolished osmotin capability but also further enhanced AD pathology by increasing SREBP2, APP, β-secratase (BACE1) expressions and the levels of toxic Aβ production, while the opposite is true for SREBP2 when silenced with its siRNA in APPswe/ind-transfected SH-SY5Y cells. Similarly, osmotin treatment also enhanced the non-amyloidogenic pathway by activating α-secratase gene i.e. ADAM10 in AMPK/SIRT1 dependent manner. These results suggest that osmotin or osmotin-based therapeutic agents might be potential candidates for AD treatment.

Disclosures: **M. Kim:** None. **S. Ali Shah:** None. **G. Yoon:** None. **M. Kim:** None. **M. Jo:** None. **M. Cho:** None.

Poster

695. Biochemical Manipulations in Animals: Prevention of Alzheimer's Disease

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 695.11/Q5

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Calmodulin-like skin protein prevents spatial learning impairment of Alzheimer's disease model mice

Authors: *S. KUSAKARI[1], M. NAWA[1], K. SUDO[2], M. MATSUOKA[1,3];
[1]Dept. of pharmacology, [2]Animal Reserch Ctr., [3]Dept. of Dermatological Neurosci., Tokyo Med. Univ., Shinjuku-ku, Tokyo, Japan

Abstract: Alzheimer's disease (AD) is the most prevalent dementia-causative neurodegenerative disorder. AD is pathologically characterized by neuronal loss, senile plaque, and neurofibrillary tangle. Three familial AD-causative genes, APP, PSEN1 and PSEN2, are identified by genetic analysis.
We previously found that a secreted neuroprotective peptide, humanin, inhibits neuronal cell death, caused by the AD-linked genes via the heterotrimeric Humanin receptor. Level of humanin in the central nervous system may be insufficient to exert a protective effect. Recently, we found that calmodulin-like skin protein (CLSP) is another physiological agonist of the Humanin receptor with an anti-cell death activity 100000-fold more potent than Humanin. CLSP is secreted from skin and related epithelial tissues, circulates in the bloodstream, and is transported to the central nervous system through the blood-brain barrier.
To investigate the effect of CLSP against the AD pathology and AD-related dementia, we generated mouse CLSP-1 transgenic mice and crossed them with the APPswe/PSEN1dE9 mice, AD model mice with the Swedish mutant gene of amyloid beta precursor protein (APP) and the presenilin-1 (PSEN1) gene lacking exon 9. It has previously established that senile plaques are deposited in hippocampus and cerebellum cortex of the aged APPswe/PSEN1dE9 mice and spatial learning was impaired in these mice. Performing the Morris water maze, we found in the current study that the constitutive co-expression of the mCLSP-1 gene prevented the impairment of spatial learning in APPswe/PSEN1dE9/CLSP-1 mice at the age of 13 months. This result suggests that CLSP behaves as a neuroprotective factor against AD in vivo.

Disclosures: **S. Kusakari:** None. **M. Nawa:** None. **K. Sudo:** None. **M. Matsuoka:** None.

Poster

695. Biochemical Manipulations in Animals: Prevention of Alzheimer's Disease

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 695.12/Q6

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: DA-9803 prevents deposition of amyloid plaques and maintains calcium homeostasis in Tg2576 mice

Authors: *G. PAGNIER[1], K. KASTANENKA[1], M. SOHN[2], S. CHOI[2], H. GO[2], B. BACSKAI[1];
[1]Massachusetts Gen. Hosp., Charlestown, MA; [2]Dong-A ST, Yongin, Korea, Republic of

Abstract: Alzheimer's Disease (AD) is a neurodegenerative disorder characterized by deposition of amyloid plaques and formation of intracellular neurofibrillary tangles, resulting in a progressive memory loss and cognitive decline. The molecular mechanisms driving these pathologies are potential targets for the treatment of AD. We studied the effects of DA-9803, a multimodal botanical cocktail in a transgenic mouse model of AD. Using multiphoton microscopy, longitudinal imaging of the senile plaques was performed and intracellular calcium was monitored. Cytosolic calcium is an indirect marker of neuronal activity and is normally tightly regulated. Our past research has shown that resting calcium is elevated in a fraction of neurites in APP transgenic mice. Thus, an effective AD treatment would restore calcium to control levels. 100 mg/kg DA-9803 was administered daily to 5 month-old Tg2576 mice via a gavage treatment for 2 months. Longitudinal imaging was performed before and during the treatment. Plaques were labeled with methoxy-XO4 while intraneuronal calcium levels were measured with the genetically encoded calcium sensor YC3.6. Chronic administration of DA-9803 prevented amyloid plaque deposition over the 2 months treatment period in these mice. Elevated calcium was detected in a subset of neurons before treatment. DA-9803 decreased the elevated neurite calcium levels to control levels over this time course. Treatment with a vehicle cocktail failed to decrease the rate of plaque deposition or restore intracellular calcium. In summary, these results demonstrate that treatment of young Tg2576 mice with 100 mg/kg DA-9803 prevents deposition of amyloid plaques and maintains calcium homeostasis in Tg2576 mice. Thus, DA-9803 treatment could have a protective effect on neuronal function and delay the progression of AD.

Disclosures: **G. Pagnier:** None. **K. Kastanenka:** None. **M. Sohn:** A. Employment/Salary (full or part-time): Dong-A ST. **S. Choi:** A. Employment/Salary (full or part-time): Dong-A ST. **H. Go:** A. Employment/Salary (full or part-time): Dong-A ST. **B. Bacskai:** None.

Poster

695. Biochemical Manipulations in Animals: Prevention of Alzheimer's Disease

Location: Halls B-H

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Program#/Poster#: 695.13/Q7

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Hassel Family Foundation

NIH F30AG048710

NIH NS085171

Title: DeltaFosB gene targets in an Alzheimer's disease mouse model: implications for hippocampal neuronal function

Authors: *G. S. STEPHENS[1], J. C. YOU[2], C.-H. FU[1], X. ZHANG[1], J. CHIN[1]; [1]Neurosci., Baylor Col. of Med., Houston, TX; [2]Neurosci., Thomas Jefferson Univ., Philadelphia, PA

Abstract: Increasing evidence suggests that seizures contribute to cognitive decline in Alzheimer's disease (AD). Therefore, understanding the mechanisms by which they do so may enable the discovery of novel therapeutic targets. Our studies of seizure-related changes in the hippocampus of human amyloid precursor protein (hAPP) transgenic mice, a mouse model of AD, have revealed that expression of the transcription factor ΔFosB is markedly increased by seizures. ΔFosB has an unusually long half-life (~8 days) and interacts with various histone modification enzymes to regulate multiple neuronal functions. Identifying targets of ΔFosB in hAPP mice may therefore provide insight into the mechanisms by which even infrequent seizures can lead to long-lasting impairments in memory. Our unbiased ChIP- and RNA-sequencing analyses have identified many putative ΔFosB gene targets in wild-type and hAPP mice. Gene clustering analyses of these targets demonstrate the potential involvement of ΔFosB in regulating pathways critical to neuronal function, including glutamatergic synapse maintenance, cytoskeletal organization, and dendritic development. Further benchtop confirmation of several notable ΔFosB targets in the hippocampus of hAPP mice reveals altered expression. Overall, our data suggest that seizure-induced ΔFosB in the hippocampus of hAPP mice critically coordinates several programs of gene expression to regulate neuronal function. This work was supported by the Hassel Family Foundation (JC), and NIH grants F30AG048710 (JCY) and NS085171 (JC).

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Poster

695. Biochemical Manipulations in Animals: Prevention of Alzheimer's Disease

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: This research was supported by a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (HI14C2173)

Title: Hyperpolarized ^{13}C-magnatic resonance spectroscopic imaging in the brain of high fat diet-fed mouse

Authors: S. LEE[1,2], S. KO[1], Y. CHOI[3], E. KIM[2,4], C. KIM[2,5], H. SONG[3], W. LEE[1], *J. LEE[1,2]; [1]Anat., Yonsei Univ. Col. of Med., Seoul, Korea, Republic of; [2]Brain Korea 21 PLUS Project for Med. Science, Yonsei Univ., Seoul, Korea, Republic of; [3]Radiology, [4]Psychiatry, [5]Pharmacol., Yonsei Univ. Col. of Med., Seoul, Korea, Republic of

Abstract: Epidemiological and clinical studies show that beta amyloid aggregation at any stage is insufficient to develop a sporadic Alzheimer's Disease (AD) which accounts for more than 85% of both sporadic and familial AD. Moreover, recent studies show that sporadic AD arises from dysregulation of brain glucose metabolism which the amyloid hypothesis does not explain. Also it is known to occur before beta amyloid plaques accumulates in the brain. In this study, we investigated the correlation between dysregulation in brain glucose metabolism and early-time pathophysiology in AD using high fat diet (HFD)-fed mouse. The mouse, to detect alteration of glucose metabolism in the AD-like mouse brain, was subjected to dynamic nuclear polarization-enhanced hyperpolarized **^{13}C-** magnetic resonance spectroscopic imaging (DNP-MRSI), following HFD for 6 months. Specifically, metabolic amounts and its speed of hyperpolarized **^{13}C**-labeled pyruvate were 1) temporally analyzed with dynamic MRS and 2) spatially analyzed with chemical shift imaging (CSI) methodology. Abnormal lactate conversion, which is similar to the Warburg Effect, from hyperpolarized 13C pyruvate was observed in the hippocampal region of HFD-fed mice. Memory function loss was confirmed with Morris Water Maze (MWM) and aggregation of beta amyloid 1-42 was found in CA1 and DG. Together, our results demonstrate that the abnormal accumulation of beta amyloid and of lactate residues through Warburg effect in the brain could play a role in the neuropathology of AD.

Disclosures: **S. Lee:** None. **S. Ko:** None. **Y. Choi:** None. **E. Kim:** None. **C. Kim:** None. **H. Song:** None. **W. Lee:** None. **J. Lee:** None.

Poster

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Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 695.15/Q9

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Tau-mediated circadian rhythm disruption and cognitive dysfunction in Alzheimer's disease mouse model

Authors: *A. KIM[1], H. SONG[2], I. MOOK-JUNG*[2];
[1]Col. of Medicine, Seoul Natl. Univ., Seoul, Korea, Republic of; [2]Seoul Natl. Univ., Seoul, Korea, Republic of

Abstract: Two pathological characteristics of Alzheimer's Disease (AD), the most common type of dementia, are Aβ senile plaques and Neurofibrillary tangles (NFTs), composed of hyperphosphorylated tau aggregation. Circadian rhythm disruption is commonly reported by patients with AD and Mild cognitive impairment (MCI). Clock genes, such as PER2, BMAL1, and CLOCK, show oscillating expression patterns in Suprachiasmatic Nucleus (SCN), the master pacemaker generating circadian rhythm. This rhythmic expression influences several physiological function including metabolism and memory formation. Moreover, some of hippocampal gene expressions are also modulated by circadian rhythm generated by SCN and show similar oscillation pattern. Thus, disruption in circadian rhythm can attributes to cognitive dysfunction in AD patients. Here, we showed that the oscillating patterns of clock genes are altered in SCN and hippocampus of AD mouse model with human tau P301L mutation. Moreover, NIH-3T3 cell lines transfected with human tau P301L mutation showed different clock genes promotor activity oscillations. Therefore, the mechanism of tau-mediated circadian dysfunction will provide a probable solution for AD patients to treat their difficulties caused by circadian rhythm disruption.

Disclosures: A. Kim: None. **H. Song:** None. **I. Mook-Jung*:** None.

Poster

695. Biochemical Manipulations in Animals: Prevention of Alzheimer's Disease

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 695.16/Q10

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Mechanisms of redox perturbation in the 2nd generation App knock-in mouse model of Alzheimer's disease

Authors: *S. HASHIMOTO, T. SAITO, T. C. SAIDO;
RIKEN BRAIN SCIENCE INSTITUTE, Wako-City, Saitama, Japan

Abstract: Objectives: Perturbed balance between oxidant and antioxidant species, such as glutathione, in brain may contribute to etiology of Alzheimer's disease (AD). A number of studies have revealed decreased levels of glutathione in the blood and CSF from AD patients. In this study we investigated the mechanisms that alter glutathione homeostasis in AD using APP knock-in (KI) mouse model (*App*$^{NLGF/NLGF}$-KI). **Methods and Results:** 12 month-old *App*$^{NLGF/NLGF}$-KI mice, carrying saturated Aβ pathology, exhibited lower glutathione levels in both blood and cortex than age matched controls. We then determined the levels of glutamate cysteine ligase catalytic subunit (GCLC), a rate-limiting enzyme in glutathione biosynthesis. Western blot and RT-PCR analyses showed a significant decrease of GCLC protein and mRNA levels in the cortex of *App*$^{NLGF/NLGF}$ mice. We next examined Nrf2 levels because Nrf2 regulates the expression of antioxidant genes and facilitates GCLC expression. Despite our hypothetical presumption, the protein levels of Nrf2 remained unchanged in *App*$^{NLGF/NLGF}$ mice. Intriguingly, *App*$^{NLGF/NLGF}$ mice exhibited higher levels of matured cortical TGF β1, which negatively regulates Nrf2-mediated GCLC expression. **Conclusions:** TGFβ1 may contribute to the decrease of glutathione and, consequently, increase oxidative stress in *App*$^{NLGF/NLGF}$ mice: TGFβ1 thus may become a target for prevention and treatment of AD.

Disclosures: S. Hashimoto: None. **T. Saito:** None. **T.C. Saido:** None.

Poster

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Program#/Poster#: 695.17/Q11

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Alzheimers related gene expression and neurosteroid production in the brains of hypogonadal kisspeptin receptor knockout mice

Authors: M. GOSS, A. R. HARVEY, *J. T. SMITH;
The Univ. of Western Australia, Perth, Australia

Abstract: Gonadal sex steroids have been implicated in the processing of β-amyloid precursor protein which generates β-amyloid (Aβ), a primary driving factor for Alzheimer's disease (AD). Neurosteroidogensis has also been evidenced in localized regions of the brain associated with AD, and has been suggested as a potential compensatory neuroprotective mechanism in response to Aβ pathology and loss of circulating sex steroids. Kisspeptin signalling controls the hypothalamic-pituitary-gonadal axis responsible for regulation of circulating sex steroid levels, however kisspeptin is also neuroprotective against Aβ toxicity, independant of its receptor, through direct binding to the Aβ peptide. We used a hypogonadal kisspeptin receptor (Kiss1r) knockout (KO) mouse model to investigate the relationship between kisspeptin signalling /sex steroid deficiency and AD-like neuropathological characteristics. Brain regions were dissected and analysed for gene expression of AD- and neurosteroidogenic-associated markers. Behavioural testing was also performed in the male cohort. Hippocampal-associated behavioural deficiencies in 6 month Kiss1r KO males were linked to increased expression of the Aβ-associated gene presenilin 1 (PSEN) within the hippocampus. Gene expression analysis of brain regions in male and female mice showed variably increasing expression of Aβ-associated genes β-amyloid cleaving enzyme (BACE), presenilin 1 (PSEN), and apolipoprotein E with age in males. Females tended to exhibit higher levels of expression of these markers than males. The neurosteroidogenic markers, steroidogenic acute regulatory protein, 3β-hydroxysteroid dehydrogenase, and cytochrome P450scc (CYP), were also generally elevated in females and increased in males with aging. Within the hypothalamus a significant ($p<0.05$) correlation between BACE and PSEN expression was found, and between BACE and CYP. The results of this experiment suggest that neurosteroid production may be acting as a compensatory neuroprotective mechanism in Kiss1r KO mice and potentially implicates kisspeptin as a significant regulator of Aβ and AD-like behaviours.

Disclosures: **M. Goss:** None. **A.R. Harvey:** None. **J.T. Smith:** None.

Poster

695. Biochemical Manipulations in Animals: Prevention of Alzheimer's Disease

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Dept. Internal Medicine intramural funds

Title: Prevention of insulin resistance in brain through mesenchymal stem cell therapy approach as potential therapeutic strategy against Alzheimer's disease.

Authors: *S. SAIEVA[1,4,5], H. S. SALLAM[1], B. TUMURBAATAR[1], R. ANZALONE[4,5], G. LA ROCCA[4,5], G. TAGLIALATELA[2,3], N. ABATE[1];
[1]Intrnl. Medicin - Div. of Endocrinol., [2]Mitchell Ctr. for Neurodegnerative Dis., [3]Neurol., Univ. of Texas Med. Br., Galveston, TX; [4]Exptl. Biomedicine and Clin. Neurosciences - Section of Human Anat., Univ. of Palermo, Palermo, Italy; [5]Inst. Euro-Mediterraneo di Scienza e Tecnologia (IEMEST), Palermo, Italy

Abstract: Compelling evidence indicates that Type 2 Diabetes Mellitus (T2DM) and Alzheimer's Disease (AD) may possibly share a common pathological origin, but the underlying mechanism(s) remains poorly understood. While insulin in the brain act as neurotrophic factor, T2DM is a known risk factor for AD and Insulin Resistance (IR - a hallmark signature of T2DM) has been extensively documented in the Central Nervous System (CNS) of AD patients. Notably, insulin plays a key-role in learning and memory, suggesting that reduced insulin signaling in the AD brain may contribute to impaired cognitive function. We developed a mouse model of T2DM that overexpresses ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1), a membrane glycoprotein that acts as an inhibitor of insulin receptor, in adipose tissue (At-ENPP1-Tg mouse) offering a unique chance to explore novel mechanistic pathways involved in IR, metabolic syndrome diabetes complications, including AD. Our initial studies showed that At-ENPP1-Tg mice display altered lipid composition of hippocampal synaptosomes, suppressed basal synaptic transmission and reduced insulin receptors. Furthermore, synapses from brain slices of At-ENPP1-Tg demonstrate increased vulnerability to Aβ oligomers, an event known to play a key role in the onset and progression of cognitive decline in AD. Adiponectin, reduced in At IR, reduces the binding of Aβ oligomers to synaptosomes of At-ENPP1-Tg mice. This initial evidence suggests the attractive possibility that targeting adipose tissue IR could be a potentially effective therapeutic strategy for AD. With this goal in mind, in the present work, we investigated the putative protective role of Wharton's Jelly-derived Mesenchymal Stem Cells (WJ-MSCs) after their transplantation in the subcutaneous adipose tissue of At-ENPP1-Tg mice. Our results show that these cells may be able to rescue the diseased At tissue and ameliorating its dysfunctional CNS impact. We are further measuring glucose and insulin level in the blood after intraperitoneal injection of glucose or insulin 12 weeks following WJ-MSCs transplantation into the At, along with tissue protein and RNA expression levels. Collectively, these studies indicate that WJ-MSCs may be used as novel therapeutic strategy to correct At IR, thus reducing associated CNS deficits.

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Poster

695. Biochemical Manipulations in Animals: Prevention of Alzheimer's Disease

Location: Halls B-H

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Program#/Poster#: 695.19/Q13

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Experimental neprilysin activation strategy for prevention of AD

Authors: *N. KAKIYA, T. SAITO, P. NILSSON, T. C. SAIDO;
RIKEN Brain Sci. Inst., Wako-Shi / Saitama, Japan

Abstract: Neprilysin is one of major amyloid-β peptide (Aβ) degrading enzymes, the expression of which decreases in the brain with aging, resulting in a metabolic imbalance of Aβ quantity. This imbalance is likely to contribute to the amyloidosis underlying sporadic Alzheimer's disease (AD). Pharmacological activation of neprilysin during aging therefore represents a potential strategy to decrease the amyloidosis and attenuate the pathological progression of AD. We have previously reported that the neuropeptide somatostatin (SST) enhances neprilysin activity. However, it still remains to be shown whether SST receptor(s) are feasible targets. To identify alternative druggable target, we first carried out an in vitro screening using mouse cortical/basal ganglia-derived neuronal co-culture system. This led to a finding of a novel promising candidate, which reduced Aβ level in the conditioned medium. Of particular importance, this candidate compound can cross the blood-brain barrier and reach the brain parenchyma after intraperitoneal injection. Thus, we investigated its in vivo effects on the neprilysin and Aβ levels in brain. We observed a significant reduction of the Aβ levels in association with an increased neprilysin expression after drug administration. We confirmed that this does not happen in neprilysin knock-out mice, indicating that neprilysin mediated the effect. We then evaluated the long-term effects, by continuously implanting pellets containing the drug under the skin of aged AD model mice (single App knock-in mice) for 90 days. We then measured cognition of the mice 2 weeks after drug administration was ceased. The drug treatment attenuated cognitive impairment observed in the AD model mice. These observations indicated that this drug-induced neprilysin activation may be applied for prevention of AD.

Disclosures: **N. Kakiya:** A. Employment/Salary (full or part-time): RIKEN BIO. **T. Saito:** A. Employment/Salary (full or part-time): RIKEN BIO. **P. Nilsson:** None. **T.C. Saido:** A. Employment/Salary (full or part-time): RIKEN BIO.

Poster

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Juvenile Diabetes Research Foundation Grant RR019963/OD010965

Title: Insulin dysregulation leads to hippocampal neprilysin downregulation

Authors: *J. MORALES-CORRALIZA[1], H. WONG[2], M. J. MAZZELLA[2], S. CHE[1], S. LEE[1], E. PETKOVA[1], J. D. WAGNER[3], S. E. HEMBY[4], S. D. GINSBERG[1], P. M. MATHEWS[1]; [1]Nathan Kline Institute-New York Univ., Orangeburg, NY; [2]Nathan Kline Inst., Orangeburg, NY; [3]Wake Forest Sch. of Med., Winston-Salem, NC; [4]High Point Univ., High Point, NC

Abstract: Objectives: To understand the mechanisms of increased Alzheimer's disease (AD) risk caused by diabetes and altered brain insulin signaling. **Methods**: Tissue from multiple brain regions of a vervet monkey model of streptozotocin-induced type 1 diabetes supported with daily insulin treatment were examined for biochemical changes in brain insulin signaling, tau protein, amyloid precursor protein (APP) and Aβ peptide, as well as changes in key enzymes that contribute to tau and APP posttranslational processing. Using primary neuronal cultures, we modeled chronic insulin dysregulation by means of prolonged insulin treatment to determine whether altered insulin signaling may directly drive AD risk in diabetics. Additionally, we manipulated insulin signaling in the brain in mice using shRNA constructs to knockdown hippocampal expression of insulin receptor substrate 1 (IRS1), a key molecule in the insulin-signaling cascade. **Results**: Regional brain analyses showed a brain-wide inhibition of IRS1, while changes in ERK1/2 phosphorylation argue for activation of this kinase, in diabetic compared to control monkeys. Increased tau phosphorylation was also seen throughout the brain in the diabetic monkeys. However, a diabetes-induced increase in Aβ levels was specific to temporal brain structures, with the greatest increase observed in the hippocampus. A

hippocampal specific decrease in the Aβ-degrading enzyme neprilysin (NEP) appears to be an important contributor to this regional Aβ increase in the diabetic monkeys. In primary hippocampal neurons, we found that prolonged insulin treatment leads to an increase in an inhibitory phosphorylation of IRS1 and, similar to the diabetic hippocampus, a reduction in NEP protein and RNA levels. Hippocampal injection of an IRS1-shRNA adeno-associated virus in wild-type mice reduced IRS1 protein levels in 3 weeks. NEP protein levels were also found to be reduced in the ipsilateral compared to contralateral hippocampus after IRS1 knockdown. **Conclusions**: In a non-human primate model of type 1 diabetes treated with exogenous insulin, reduced NEP expression in the hippocampus correlates with biochemical alterations in the insulin-signaling pathway that has been described as "brain insulin resistance". Direct manipulation of insulin levels and the key insulin-signaling mediator IRS1 supports the conclusion that disruption of insulin signaling leads to the reduced NEP expression seen in the diabetic hippocampus. Our findings are consistent with the idea that brain insulin resistance increases the risk for AD both by increasing brain Aβ levels and by altering tau phosphorylation.

Disclosures: J. Morales-Corraliza: None. **H. Wong:** None. **M.J. Mazzella:** None. **S. Che:** None. **S. Lee:** None. **E. Petkova:** None. **J.D. Wagner:** None. **S.E. Hemby:** None. **S.D. Ginsberg:** None. **P.M. Mathews:** None.

Poster

695. Biochemical Manipulations in Animals: Prevention of Alzheimer's Disease

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 695.21/R1

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH 1R01AG042890 (GT)

Title: Near infrared light reduces amyloid beta oligomer-driven synaptic dysfunction.

Authors: *M. M. COMEROTA[1], G. TAGLIALATELA[2];
[2]Mitchell Ctr. for Neurodegenerative Diseases, Dept. of Neurol., [1]Univ. of Texas Med. Br., Galveston, TX

Abstract: Alzheimer's disease (AD), the most common age related neurodegenerative dementia, is characterized by deposits of aggregated amyloid beta (Aβ) plaques and neurofibrillary tangles comprised mainly of hyper-phosphorylated tau protein. However, it is believed that the association of small toxic Aβ oligomers with synapses contributes to the cognitive decline that is associated with AD. This association results in dysfunctional synaptic morphological and physiological changes including retraction of synaptic spines and reduced long term potentiation

(LTP). Because current pharmaceutical therapeutic options have limited efficacy and the prevalence of AD is rising, the development of alternative treatments are imperative. Near infrared (NIR) light treatment (600-1000 nm) is a novel noninvasive therapeutic strategy that has been suggested as an effective option for the treatment of AD. Notably, it has been reported that NIR light treatment on APP/PS-1 transgenic mice induced a reduction of Aβ plaque load and improved memory function. However, the effect of NIR light on the most toxic form of Aβ, oligomers, and their impact on synapses remained undescribed. In the present study, we investigated the presence of Aβ oligomers at synapses and the susceptibility of synapses to Aβ binding (an important event linked to Aβ-driven synaptic disruption and memory deficits) after a NIR light treatment at 670 nm (90 sec a day for 4 weeks). We further examined the changes in the synaptic morphological and physiological properties after such NIR light treatment. We found that after NIR light treatment, the amount of $A\beta_{1-42}$ was significantly reduced at synapses of 6 month old APP transgenic mice (Tg2576) that was paralleled by an increased retention of long term potentiation induction. We further found that the synapses of wild type mice treated with NIR light showed a reduction in *ex vivo* Aβ oligomer binding. Collectively these results indicates that NIR light, in addition to reducing levels of Aβ oligomers, further promotes synaptic resistance to Aβ oligomer binding thus alleviating the ensuing synaptic impairments. This study thus provides evidence that NIR can effectively reduce Aβ driven synaptic dysfunction, thus furthering light therapy as a viable treatment for AD.

Disclosures: M.M. Comerota: None. **G. Taglialatela:** None.

Poster

695. Biochemical Manipulations in Animals: Prevention of Alzheimer's Disease

Location: Halls B-H

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Program#/Poster#: 695.22/R2

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: National Natural Science Foundation of China (81271476) to Feng Li

The 111 Project, China (B13037) to Feng Li

Science and Technology Research Foundation of Guangzhou, China (201300000154) to Feng Li

Title: New selective LXR modulators as potent drugs for treatment of Alzheimer's disease

Authors: Y. YANG[1], L. WANG[2], C. FIDELIS[1], J. HUANG[1], Z. LIANG[1], M. KANG[1], Y. KUANG[1], F. JIA[1], M. XIE[1], S. ULLAH[1], *J. S. SHUMSKY[4], W.-J. GAO[4], B. JIANG[2], F. LI[1,3];

[1]Neurobio. and Anat., Zhongshan Sch. of Medicine, Sun Yat-Sen Univ., Guangzhou, China; [2]Physiol., [3]Guangdong Provincial Key Lab. of Brain Function and Dis., Zhongshan Sch. of Medicine, Sun Yat-Sen University, China, Guangzhou, China; [4]Neurobio. and Anat., Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: Cholesterol metabolism may play a role in modulating Alzheimer's disease (AD) risk and pathogenesis. Recent studies suggest that apoE receptors and very low density lipoprotein (VLDL) receptors may be implicated in memory and neurodegenerative disorders like AD. VLDL receptor-deficient mice have a moderate deficit in long term potentiation (LTP). Liver X receptors (LXRβ and LXRα) are nuclear oxysterol receptors and metabolic sensors initially found to regulate cholesterol metabolism and lipid biosynthesis. The synthetic LXR agonist T0901317 inhibits APP processing and lowers Aβ in the brains of APP transgenic mice possibly via ABCA1 (ATP-binding cassette transporter A1) dependent mechanism, and activation of LXR/ABCA1 pathway in the brain can positively affect Aβ metabolism. Thus, LXR agonists may provide a potential pharmacological strategy for AD treatment. However, LXR agonists target both LXRβ and LXRα to elevate hepatic and serum trigylceride levels. Therefore we tested the efficacy of subtype-specific LXR agonists. We synthesized 41 compounds of flavonoids using the typical flavone and chalcone skeletons as the templates for structure-diversity expanding, 37 compounds of them are new compounds. We confirmed that some compounds, particularly compound 19, activate LXRβ rather than LXRα, and upregulate the expression of ABCA1 and/or apoE. Further, these compounds facilitate intracellular Aβ clearance. The 7 month-old APP/PS1 transgenic mice were injected with compound 19 (10 mg/kg, IP, once every other day for 3 months), with positive control mice being given T0901317 (25 mg/kg, by gavage daily). We found that compound 19 significantly reduced Aβ and senile plaques in the brain, and more importantly it had no effect in serum or hepatic triglyceride levels compared with those given T0901317. Moreover, we provided the first evidence that compound 19 significantly enhanced LTP in CA1 (169.2±7.1% in control AD mice vs. 212.8±8.3% in AD mice with compound 19). Our findings suggest that compound 19 or its analogues may serve as new selective LXR modulators for treatment of Alzheimer's disease.

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Poster

695. Biochemical Manipulations in Animals: Prevention of Alzheimer's Disease

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: ARC DP130101932

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NHMRC APP1037746

NHMRC 1003150

Title: Scanning ultrasound as a treatment tool of proteinopathies including Alzheimer disease

Authors: *J. GOETZ, G. LEINENGA, R. NISBET, A. VAN DER JEUGD, R. HATCH; Queensland Brain Inst., The Univ. of Queensland, Brisbane (St Lucia Campus), Australia

Abstract: Background: Treatment strategies for diseases are hampered by the fact that the blood-brain barrier (BBB) establishes an efficient barrier for therapeutic agents. We have recently shown that scanning ultrasound (SUS) allows microglial-mediated clearance of amyloid-beta in APP mutant APP23 mice and restores memory functions to wild-type levels (Leinenga and Götz, Science Transl Med 2015; Leinenga et al., Nat Rev Neurol 2016). While no damage was detected by histology, the effect of SUS on neuronal firing or complex neuronal morphology was not studied. We also determined whether SUS treatment is safe long-term and whether it reduces the intracellular tau pathology that characterizes Alzheimer's disease, in addition to amyloid deposition. **Methods:** For the safety study, we treated C57BL/6 wild-type mice both acutely and chronically with either one or several weekly SUS treatments. Upon sacrifice, the electrophysiological properties and morphology of hippocampal neurons of treated mice were analysed. To assess the efficacy and drug-delivering ability of SUS in tau models, we established four groups, using a novel anti-tau antibody which was injected weekly over four weeks, either on its own, or together with SUS. A third group used SUS only, and a fourth was the anaesthesia control group. As an experimental model, P301L tau transgenic pR5 mice were used. **Results:** Whole-cell patch-clamp electrophysiology revealed that SUS does not have any harmful effects on neuronal firing, as treatment (even three months after six weekly treatments) did not alter action potential firing. Further SUS treatment prevented age-related loss of synaptic spines. A histological and biochemical analysis of the pR5 tau transgenic mice revealed that SUS as well as the employed antibody ameliorate the tau pathology that characterizes the pR5 mice, and that there are synergistic effects, presenting opportunities to use SUS for drug delivery. **Conclusions:** Our study demonstrates that long-term treatment of mice with ultrasound is safe using electrophysiology and maintains synaptic structure. The study further suggests that SUS is a method that benefits diseases with protein aggregates more generally, whether they are intra- or extracellular.

Disclosures: J. Goetz: None. **G. Leinenga:** None. **R. Nisbet:** None. **A. van der Jeugd:** None. **R. Hatch:** None.

Poster

695. Biochemical Manipulations in Animals: Prevention of Alzheimer's Disease

Location: Halls B-H

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Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

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Title: Neurochemical changes in the anemic neonatal mouse hippocampus and prefrontal cortex following erythropoietin therapy

Authors: *D. WALLIN, I. TKAC, M. K. GEORGIEFF;
Univ. of Minnesota, Minneapolis, MN

Abstract: Background: Alterations to brain energy and phospholipid metabolism have been shown in the hippocampus of a neonatal mouse model of phlebotomy-induced anemia (PIA). Other brain regions such as the prefrontal cortex that are developing during this time period are also at risk for metabolic abnormalities. These changes may be due to hypoxia, iron deficiency (ID), or both. Erythropoietin is commonly used in the neonatal intensive care unit (NICU) and could alleviate the hypoxic effects of PIA on the brain without the risk of a red blood cell transfusion. However, erythropoietin could also exacerbate brain ID by prioritizing the neonate's limited iron reserves to the growing red blood cell mass. Objective: Our objective was to use a mouse model of PIA that is both physiologically and developmentally relevant to study the effect of erythropoietin treatment in the hippocampus and prefrontal cortex of anemic neonatal mice. Methods: Neonatal mice were bled starting at postnatal day (P)3 to a hematocrit of less than 25% and then treated with recombinant human erythropoietin (RhEpo) to achieve a hematocrit above this threshold. Metabolic profiles of PIA, PIA and RhEpo treated, and nonbled controls were obtained through in vivo ^{1}H NMR spectroscopy at 9.4T in the hippocampus and prefrontal cortex. Results: Lactate was significantly increased in the PIA mouse hippocampus as compared to nonbled control. An increase was also found in the prefrontal cortex. Lactate concentrations in both structures were not different between PIA mice and PIA mice given RhEpo treatment. Glutamate is increased in both structures in the PIA mice (trending in prefrontal cortex), and RhEpo treatment begins to normalize these concentrations. A similar trending effect is found for GPC+PCho in both structures and for GSH within the prefrontal cortex. Conclusions: RhEpo

treatment did not alleviate the bioenergetics alterations due to PIA, indicating that these alterations may primarily be due to brain ID and not anemia. Concentrations that were altered with anemia and normalized with RhEpo treatment such as glutamate appear to be due to anemia, rather than brain ID. Better understanding of the underlying neurochemical alterations during anemia and the long-term risks posed will lead to more informed decisions regarding phlebotomy (number of blood draws), RBC transfusions and the use of RhEpo by clinicians caring for preterm infants in the NICU.

Disclosures: **D. Wallin:** None. **I. Tkac:** None. **M.K. Georgieff:** None.

Poster

695. Biochemical Manipulations in Animals: Prevention of Alzheimer's Disease

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Program#/Poster#: 695.25/R5

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: R41AG043243

ADDF20140803

Title: Identification of selective sigma 1R ligand EPGN644 for evaluation in Alzheimer's disease

Authors: ***S. RAVULA**[1], F. C. TUCCI[1], G. BEATON[1], C. BORSAN[1], R. DAGAR[1], S. KIM[2], D. DALWADIDI[2], J. A. SCHETZ[2];
[1]Epigen Biosci. Inc,, San Diego, CA; [2]Univ. of North Texas Hlth. Sci. Ctr., Fort Worth, CA

Abstract: Alzheimer's Disease (AD) is a significant public health problem yet available treatments offer limited benefit and none are truly disease-modifying. Cognitive decline is slower in AD patients with higher levels of brain BDNF, while interference with BDNF trafficking and secretion is associated with an increased risk of AD. Lead discovery identified several novel small molecules that facilitate BDNF secretion via their high affinity and selective interactions with the Sigma-1 (S1R) suggesting that they would be beneficial in AD by making neurons more resilient to inflammatory stress and promoting neurogenesis. EPGN644 is one of these compounds and it has pharmacokinetic properties suitable for conducting further studies in rodent models.

Disclosures: **S. Ravula:** A. Employment/Salary (full or part-time): Epigen Biosciences Inc. **F.C. Tucci:** A. Employment/Salary (full or part-time): Epigen Biosciences Inc. **G. Beaton:** A. Employment/Salary (full or part-time): Epigen Biosciences Inc. **C. Borsan:** A.

Employment/Salary (full or part-time): Epigen Biosciences Inc. **R. Dagar:** A. Employment/Salary (full or part-time): Epigen Biosciences Inc. **S. Kim:** A. Employment/Salary (full or part-time): University of North Texas Health Science Center. **D. Dalwadidi:** A. Employment/Salary (full or part-time): University of North Texas Health Science Center. **J.A. Schetz:** A. Employment/Salary (full or part-time): University of North Texas Health Science Center.

Poster

695. Biochemical Manipulations in Animals: Prevention of Alzheimer's Disease

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 695.26/R6

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: ARUK Grant PPG2012B-21

Title: Chronic administration of imidazoline ligands improves memory deficits and reduces neuroinflammation in a mouse model of Alzheimer's disease

Authors: *N. MIRZAEI, A. BIRCH, L. KATSOURI, D. NUTT, M. SASTRE;
Imperial Col. London, London, United Kingdom

Abstract: An accumulating body of evidence supports the use of imidazoline type-2 (I2) receptor ligands as a potential avenue for therapeutic intervention in Alzheimer’s disease (AD). These ligands seem to be neuroprotective by reducing cell death, neuroinflammation and the release of neurotoxic substances such as glutamate.
To investigate this further, 6 month old female 5XFAD mice and wild-type controls (n=8 per group) were treated with 5 $mg.kg^{-1}$ BU224 twice a day for a period of 10 days. At this age, 5XFAD mice show severe memory impairment, amyloid deposition and neuronal loss. BU224-treated 5XFAD mice showed a significant increase in exploring the displaced object on testing day, compared to both training day and vehicle-treated controls. Fear conditioning testing also revealed that both hippocampal-dependent and -independent memory functions in the BU224-treated 5XFAD mice were also improved following learning by association. Subsequently, mouse brains were perfused and used for immunohistochemical assessment and protein quantification. GFAP staining was performed and showed that BU224 treatment increases the levels of GFAP expression, as has been published before for other imidazoline ligands (Olmos et al., 1994; Alemany et al., 1995). In hippocampal and cortical homogenates, BU224 induced a decrease in the levels of certain cytokines such as IL-1β and TNF-α measured by ELISA, suggesting an anti-inflammatory effect.
In conclusion, our preliminary data indicate that chronic treatment with BU224 improves

memory and cognitive performance and reduces inflammation, at a severe stage of AD pathology. Therefore, I2-imidazoline drugs could have potential benefits for AD patients.

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Poster

695. Biochemical Manipulations in Animals: Prevention of Alzheimer's Disease

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Gebert Rüf foundation (grant GRS-002/13)

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Mats Paulsson Foundation for research

Title: Gastrointestinal dysbiosis in the development of alzheimer disease

Authors: ***T. HARACH**[1], N. MARUNGRUANG[2], F. FÅK[2], N. DUTILLEUL[1], V. CHEATHAM[1], K. MCCOY[3], J. NEHER[4], M. JUCKER[4], T. LASSER[1], T. BOLMONT[1]; [1]EPFL STI IMT LOB, EPFL, Lausanne, Switzerland; [2]Food for Hlth. Sci. Centre, Lund Univ., Lund, Sweden; [3]Mucosal Immunol. Lab. Dept. of Clin. Res., Univ. of Bern, Bern, Switzerland; [4]German Ctr. for Neurodegenerative Dis. (DZNE), Tübingen, Germany

Abstract: Alzheimer disease is the leading cause of dementia in western societies featuring huge societal and personal cost and burden. To date, there is no early diagnosis or cure for this devastating neurodegenerative disorder. Gastrointestinal microbiota is key player to many physiological processes including nutrition, inflammation, vitamins synthesis, drug processing, but also defense against pathogens. A growing body of evidence suggests that gastro-intestinal microbiota impacts on brain disorders such as autism and Parkinson’s disease. However, the role of the intestinal microbiota in AD has never been investigated. Herein, we showed that sequencing bacterial 16S rDNA extracted from fecal samples of transgenic CONVAPPPS1 and wild type mice revealed major shifts in the gut microbiota composition at both phylum and genus level. Furthermore the absence of intestinal microbiota in the germ free GFAPPPS1 transgenic mice was sufficient to significantly decrease cerebral Abeta amyloid pathology. Not only the biochemical levels of Aβ but also the extent of congophilic Aβ plaques was consistently decreased in the brains of APPPS1 transgenic mice without gut microbiota. In contrast, whereas colonization of GFAPPPS1 mice with microbiota from transgenic CONVAPPPS1 mice increases

AD pathology, colonization of GFAPPPS1 mice with Altered Schaedler Flora did not increase amyloidosis in GFAPPPS1 mice. Altogether, our results indicate that gastro-intestinal dysbiosis is involved in the development of AD and supports the view that neurodegenerative disorders may be tackled by gastro-intestinal modulation.

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Poster

695. Biochemical Manipulations in Animals: Prevention of Alzheimer's Disease

Location: Halls B-H

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: GAUK 636316

GACR 13-02154S

GACR P304/12/G069

Title: Astrocyte volume regulation during the progression of Alzheimer's disease

Authors: D. KOLENICOVA[1,2], P. HONSA[1], J. KRISKA[1], D. KIRDAJOVA[1], L. VALIHRACH[3], M. KUBISTA[3], *M. ANDEROVA[1,4];
[1]Inst. Exper Med. ASCR, Prague 4, Czech Republic; [2]Fac. of Science, Charles University,, Prague, Czech Republic; [3]Lab. of Gene Expression, Inst. of Biotech. - Biocev, Vestec, Czech Republic; [4]2nd Fac. of Medicine, Charles Univ., Prague, Czech Republic

Abstract: Astrocytes are deeply involved in the maintenance of extracellular brain homeostasis and failure of their homeostatic functions may contribute to the progression and outcome of neuropathological processes, including Alzheimer’s disease (AD). Recent evidence suggests that early stages of AD are accompanied by astrocytic atrophy and by a dis-balance in neurotransmitter homeostasis, which results in neuronal death, and also in an increased uptake of osmotically active substances, predominantly provided by astrocytes, which leads to marked volume changes in these cells. Here, we aimed to elucidate to what extent the progression of AD affects astrocyte volume changes and their regulatory volume mechanisms in response to various pathological stimuli, such as hypoosmotic stress or increased extracellular K^+ concentration (50 mM) in the mouse hippocampus using three-dimensional confocal morphometry *in situ*. We quantified the changes in total cell volume as well as the contribution of two compartments, i.e.,

cell processes and the cell soma in the hippocampal astrocytes from 3 months- (3M), 9 months- (9M) and 12 months old mice. In order to visualize individual astrocytes we used EGFP/GFAP mice (controls), in which astrocytes are fluorescently labeled and enerated new transgenic mice by crossbreeding GFAP/EGFP mice, with triple transgenic mice, in which amyloid precursor protein, presenilin and tau are mutated (3xTgAD mice). In addition, a single cell RT-qPCR profiling was carried out to reveal possible differences in the expression profiles of astrocytic ion channels/transporters that participate in maintaining ionic/neurotransmitter homeostasis. Three-dimensional confocal morphometry revealed comparable swelling of hippocampal astrocytes from 3M old controls and aged-matched GFAP/EGFP/3xTgAD mice in response to hypo-osmotic stress, however in astrocytes of 9M- and 12M old GFAP/EGFP/3xTgAD mice a slower/smaller swelling was observed when compared to that of controls. After astrocyte exposure to elevated K^+ we detected similar slower/smaller astrocytic swelling already at 3M old GFAP/EGFP/3xTgAD mice. A marked difference in ability of astrocytes to regulate their volume was detected between 12M old GFAP/EGFP/3xTgAD mice and aged matched controls. Interestingly, there were no differences in astrocyte volume regulation during physiological aging.

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Poster

696. Genetics and Epigenetics of Alzheimer's Disease and Related Models

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Program#/Poster#: 696.01/R9

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: AG043375

AG014449

AG017617

Title: Hippocampal gene expression patterns in a mouse model of Down syndrome (Ts65Dn) following maternal choline supplementation (MCS)

Authors: *M. J. ALLDRED[1,4], H. M. CHAO[1,4], S. LEE[2], J. BEILIN[1], E. PETKOVA[3,5], S. D. GINSBERG[1,4,6];
[1]Ctr. for Dementia Res., [2]Ctr. for Biomed. Imaging and Neuromodulation, [3]Child Psychiatry,

Nathan Kline Inst., Orangeburg, NY; [4]Psychiatry, [5]Child and Adolescent Psychiatry, [6]Neurosci. & Physiol., New York Univ. Langone Med. Ctr., New York, NY

Abstract: Down syndrome (DS) is the most frequent genetic cause of intellectual disability (ID) with an increasing prevalence in the USA, with current estimates of 1 in 691 live births. Individuals with DS have system-wide impairments, notably in the central nervous system, with ID and decreased cognitive function seen by impairments in hippocampal learning and memory, degeneration of basal forebrain cholinergic neurons (BFCNs), and language and communication skills. Individuals with DS also develop the pathological hallmarks of Alzheimer's disease (AD) early in mid-life, including senile plaques, neurofibrillary tangles, and early endosomal abnormalities. DS mouse models, including the Ts65Dn mouse, are trisomic for a segment of mouse chromosome 16 (MMU16) orthologous to human chromosome 21 (HSA21), and exhibit ~55% gene conservation of protein-encoding genes between MMU16 and HSA21. This model recapitulates several critical components of DS/AD, including cognitive dysfunction and BFCN degeneration, providing a platform for mechanistic assessments for translation to humans. We used the Ts65Dn mouse model to determine gene expression changes associated with DS/AD and test maternal choline supplementation (MCS) as a treatment modality. We examined gene expression changes in CA1 pyramidal neurons at two distinct timepoints, at 4-6 months (pre-BFCN degeneration) and 10-12 months (post-BFCN degeneration), postulating that MCS will improve cognition and could delay the septohippocampal degeneration seen in Ts65Dn offspring. 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 at both timepoints indicate that MCS has long-term positive effects on gene regulation in Ts65Dn mice. Several classes of transcripts showed significant alterations due to MCS treatment including neurotrophin receptors, AD-related genes, synaptic-related markers, and cell death genes in both genotypes examined, presumably showing benefits of this early intervention. We hypothesize that long-term effects of MCS treatment in both Ts65Dn and normal disomic (2N) littermates will highlight pathways and mechanisms that may be sites of intervention for cognitive decline and neurodegeneration. Moreover, these may help elucidate the link between choline requirements throughout neurodevelopment and cognitive maturation.

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Poster

696. Genetics and Epigenetics of Alzheimer's Disease and Related Models

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant 1k22NS094591

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Brain and Behavior Grant 22732

Title: Functional characterization of ank2 mutations in asd

Authors: *J. C. GARZA[1,2,3], T. PETRYSHEN[1,2,3];
[1]Ctr. for Human Genet. Research, Psychiatry, Massachusetts Gen. Hosp., Boston, MA; [2]Stanley Ctr. for Psychiatric Res., The Broad Inst. of Harvard and MIT, Cambridge, MA; [3]Psychiatry, Harvard Med. Sch., Boston, MA

Abstract: Current estimates suggest autism spectrum disorder (ASD) effects as many as 1 in 68 children. ASD is a developmental neurological disorder characterized by abnormal cognition, inhibition of social behaviors and onset of focused or isolated behaviors. Although the underlying causes are not clearly known, recent large-scale genomic studies have identified candidate genes involved in synaptic transmission and brain development that are highly associated with ASD patients. Mutations found in the candidate genes were de novo and were not a result of inherited mutations. Among the risk genes identified, ANK2 is repeatedly found in large-scale exome sequencing studies. Loss of function missense mutations identified at specific amino acid residues were shown to be highly associated with autistic patients. Ankyrin-2 is a membrane associated scaffolding protein that plays an integral role in protein localization and axon guidance and growth. Thus, any disruption to ankyrin function may have critical consequences for synaptic function and neurotransmission. Neuroanatomical studies in living patients and postmortem tissue have revealed that autistic patients have abnormal connectivity within their brain and altered development of key brain structures involved in executive function. Therefore, it is important to identify and dissect the mechanisms underlying these impairments. To address this, genome editing techniques were used to evaluate how mutations in ANK2 affect development of cultured neurons and ultimately how these mutations influence brain development and the onset of ASD-like behaviors in mice. Clustered regularly interspaced short palindromic repeat (CRISPR) guide RNA were designed to target ANK2 in cultured human neural progenitor cells. Cas9 nickase and guide RNA were cloned into lentiviral vectors to facilitate delivery into cultured cells and introduce the point mutations in the ANK2 gene. After verification of the mutation, the positive cells were used to evaluate the effects on neuronal

growth, differentiation and architecture of the cytoskeleton. Taken together, this information will advance our understanding of the molecular, cellular and neural network impairments underlying ASD.

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Poster

696. Genetics and Epigenetics of Alzheimer's Disease and Related Models

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Program#/Poster#: 696.03/R11

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: A specific transcript of SNX19 (sorting nexin 19) contributes to schizophrenia risk

Authors: *L. MA[1], Q. CHEN[1], A. JAFFE[1], M. LI[1], R. TAO[1], C. LI[1], J. SHIN[1], Y. JIA[1], N. BRANDON[2], A. CROSS[2], T. HYDE[1,3], J. KLEINMAN[1,4], D. WEINBERGER[1,4,3,5,6], R. STRAUB[1];
[1]Lieber Inst. for Brain Develop., Baltimore, MD; [2]AstraZeneca Neurosci. Innovative Medicines and Early Developmental Unit, Cambridge, MA; [3]Neurol., [4]Psychiatry and Behavioral Sci., [5]Neurosci., [6]The McKusick Nathans Inst. of Genet. Med., Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Genome-wide association studies (GWAS) have identified many genetic variants associated with risk for schizophrenia. Here we investigate the genomic region flanking rs10791097, the 16th ranked SNP in the latest Psychiatric Genomics Consortium (PGC) GWAS for schizophrenia, a risk variant which appears to be unique to the disorder. We performed RNA sequencing (RNAseq) using 495 dorsolateral prefrontal cortex (DLPFC) samples from the Lieber Institute collection of postmortem human brains, and tested for association between the genotypes at schizophrenia risk variants and expression, to detect “expression quantitative trait loci” (eQTLs). Genotype at rs10791098 (an LD proxy for rs10791097, r-squared=1) was associated with the expression of the entire SNX19 gene (p=0.0014), as assayed by RNAseq of the DLPFC from 188 adult Caucasians. We then analyzed the two more transcript-specific features of expression - exons and the splice junctions between them, and found that the rs10791098 GWAS risk allele (A) was strongly positively associated with expression of the junction between exons 8 and 10 (n=108; Junc8.10 p=3.40e-9) whereas it is not associated with expression of the junction between exons 8 and 9 (n=108; Junc8.9 p=0.538). Expanding the sample to include African American cases and controls and Caucasian cases yielded much stronger results (n=372; Junc8.10 p=1.55e-29, Junc8.9 p=0.0415). Interestingly, only 46% of the 495 postmortem DLPFC samples had Junc8.10 read counts > 0. RNAseq results were confirmed

by qPCR in DLPFC (n=79; Junc8.10 p=2.23e-7, Junc8.9 p=0.240) and in hippocampus (n=44; Junc8.10 p=8.96e-8, Junc8.9 p=0.710). Junc8.10 read counts measure primarily the predicted transcript XM_005271546, which we validated by end-to-end PCR followed by sequencing of clones. Junc8.9 read counts measure primarily the “full length” SNX19 transcript NM_014758. GTEx RNAseq data from cortex showed a similar pattern of association - strong with Junc8.10 (n=95; Junc8.10 p=2.05e-8) and weak or absent with full length transcript (p=0.0419), which was also evident in hippocampus (n=85; Junc8.10 p=3.74e-4, Junc8.9 p=0.756), and other brain regions. Finally, we found additional variants (rs34529622 and rs3831404) nearby that are in only partial linkage disequilibrium with rs10791098 but are also strongly associated with both schizophrenia and specific expression of Junc8.10 in postmortem brain. We characterized a truncated transcript of SNX19, and postulate that a critical element of the molecular mechanism of schizophrenia risk from this GWAS locus is initiated by its increased expression.

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Poster

696. Genetics and Epigenetics of Alzheimer's Disease and Related Models

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 696.04/R12

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Bergen Medical Research Foundation grant

Title: Association between amyloid-beta precursor protein (APP) genetic variant and cognitive abilities

Authors: O. NIKOLAIENKO[1], C. MYRUM[1], *C. R. BRAMHAM[2], J. HAAVIK[1], T. ZAYATS[1];
[1]Univ. of Bergen Dept. of Biomedicine, Haukeland Univ. Hospital, and K.G. Jebsen Ctr. for research on Neuropsychiatric Disorders, Bergen, Norway; [2]Univ. of Bergen, N-5009 Bergen, Norway

Abstract: Individual differences in general cognitive ability are key aspects of important life outcomes including health, longevity, education, and occupation. Cognitive function is substantially heritable and thought to have a polygenic nature. Here we focused on a set of genes functionally linked to the Arc/Arg3.1 gene (ARC complex), a key regulator of long-term synaptic plasticity, learning, and memory formation. We tested for an enrichment of association

signals with intelligence, as measured with the Weschler Intelligence Scale for Children (WISC-III) in a sample of 8.5-year-old children from the Avon Longitudinal Study of Parents and Children (ALSPAC). Permutation-based family-wise correction was applied to account for multiple testing. As Alzheimer's disease (AD) shares genetic etiology with cognitive functioning, signals surviving the correction for multiple testing were further assessed in a large case-control sample of AD (17,008 cases and 37,154 controls). Tentative association was observed between the functional gene set of ARC complex and verbal as well as total IQ measures (p = 0.027 and 0.041, respectively) in ALSPAC, with the strongest association observed for rs2830077 located within APP gene (empirical p = 0.018). This association was confirmed in the AD sample (p = 2.76E-03). Potential functional significance of this SNP was examined in silico in RegulomeDB, and further assessed using electrophoretic mobility shift assay and luciferase assays. eQTL analyses of rs2830077 showed significant alteration of APP expression (p = 2.19E-09 in the whole blood tissue).

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Poster

696. Genetics and Epigenetics of Alzheimer's Disease and Related Models

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: JPND Grant EPI-AD

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ISAO Grant 07551

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ISAO Grant 12530

Title: Differential miR-137 expression and methylation in Alzheimer's Disease

Authors: *A. IATROU[1], R. G. SMITH[2], R. LARDENOIJE[1], J. A. Y. ROUBROEKS[1,2], D. MASTROENI[1,3], P. D. COLEMAN[3], B. P. F. RUTTEN[1], K. LUNNON[2], G. KENIS[1], D. L. A.

VAN DEN HOVE[1,4];
[1]Dept. of Psychiatry and Neuropsychology, Sch. for Mental Hlth. and Neuro, Maastricht Univ., Maastricht, Netherlands; [2]Univ. of Exeter Med. Sch., Exeter, United Kingdom; [3]ASU-Banner Neurodegenerative Dis. Res. Ctr., Arizona State Univ., Tempe, AZ; [4]Dept. of Psychiatry, Psychosomatics and Psychotherapy, Univ. of Wurzburg, Wurzburg, Germany

Abstract: MicroRNAs (miRNAs) are small noncoding RNAs that are primarily involved in the posttranscriptional control of gene expression. Recently, their critical role in neurodevelopment and aging has gained momentum, associating them with neuropsychiatric disorders. Common genetic variants as well as single nucleotide polymorphisms (SNPs) in miR-137 have been associated with severe cognitive impairments in schizophrenia. Moreover, in the mature brain, miR-137 has a functional role in presynaptic vehicle release as well as synaptic strength within the hippocampal area. As such, we hypothesized that dysregulation in miR-137 expression is associated with Alzheimer's Disease (AD). Further, based on the well-documented role of DNA methylation in AD, we investigated whether differential miR-137 expression is regulated by this epigenetic modification. We thus examined the expression and methylation patters of miR-137 in middle temporal gyrus (MTG) tissue of AD patients and non-demented controls, by means of PCR and the Illumina Infinium 450K Beadarray, respectively. Our novel findings show significantly decreased miR-137 expression in the MTG of AD patients in comparison to controls. Additionally, we detected a reduction in the methylation levels of the same samples, showing a small, but significant correlation with expression levels. This study investigated the effect of dysregulated expression and methylation of miR-137 in AD and is the first one attempting to show that expression changes of this miRNA are epigenetically regulated. Our perspective objective is to investigate differential expression and methylation patterns of miR-137 in brain structures associated with the symptomatology of the most incipient stages of AD, namely brainstem nuclei. Further, we aim to elucidate whether a 15bp variable number tandem repeat (VNTR) that regulates miR-137 expression is associated with unique methylation patterns and whether its length can predict cognitive decline in a longitudinal cohort of individuals suffering from mild cognitive impairment (MCI).

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Poster

696. Genetics and Epigenetics of Alzheimer's Disease and Related Models

Location: Halls B-H

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Program#/Poster#: 696.06/R14

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Neuron-specific methylome analysis reveals epigenetic regulation of BRCA1 against Aβ-induced DNA damage in Alzheimer's disease brain

Authors: *T. MANO[1], K. NAGATA[2], S. MURAYAMA[3], S. C. TAKAOMI[2], S. TSUJI[1], A. IWATA[1];
[1]Grad. Sch. of Medicine, The Univ. of Tokyo, Tokyo, Japan; [2]Lab. for Proteolytic Neuroscience, RIKEN BSI, Wako, Japan; [3]Dept. of Neuropathology, Tokyo Metropolitan Geriatric Hosp., Tokyo, Japan

Abstract: [Background] We performed neuron-specific methylome analysis of AD brains and found profound hypomethylation in *BRCA1* promoter region. BRCA1 is known to play an important role in DNA repair and its mutation is risk factor of hereditary breast cancer, while it has been seldom studied in neuroscience. We analyzed *in vitro* and i*n vivo* models of AD
[Objective] Tto elucidatexamine the role of BRCA1 in Alzheimer's disease pathomechanism, we analyzed cellular and mice models..
[Methods] We first analyzed the effect of Aβ on BRCA1 expression and DNA damage in N2a swe.10 cells harboring mutant APP transgene with Swedish mutation. We further analyzed tested BRCA1 expression pattern, and solubility of BRCA1, and, the balance between DNA damage and repairfragmentation level of BRCA1 in post-mortem AD, 3× ×Tg-AD and APP/PS1 mice brains . We also analyzed the effect of Aβ on BRCA1 expression in APP swe. 10 cells.
[Results] N2a swe.10 cells showed increased soluble BRCA1 expression which was attenuated at the presence of γ-secretase inhibitor. They were positive for DNA double strand break (DSB) marker γ-H2ax but showed no detectable DNA fragmentation. BRCA1 was mislocalized at the cytoplasm in neuronal cells of AD and 3× ×Tg-AD brains and was found mostly at detergent insoluble fractions. However, in APP/PS1 mice it was only found at soluble fraction and cytoplasmic BRCA1 was not observed was not mislocalized. In Braak stage 3 AD brains, cytoplasmic staining of BRCA1 was limited to found only at the hippocampal CA1 region, while at Braak stage 5, they were positive beyond entorhinal through neocortex. APP swe.10 cells was positive for DNA double strand break (DSB) marker γH2ax but shoed no detectable DNA fragmentation. They showed increased BRCA1 expression which was attenuated at the presence of γ-secretase inhibitor. Compared Comparing between 3× ×Tg-AD and APP/PS1 mice, positive DNA fragmentation was only observed in 3× ×Tg-AD brains. Promoter region of *Brca1* in 3× ×Tg-AD mice was hypomethylated compared to the wild type mice.
[Conclusions] Aβ induces DNA DSB and the cell copes with the damage by up-regulating BRCA1 through epigenetic mechanism. However, in the presence of aggregated aggregated tau, BRCA1 can no longer function properly and DSB is accelerated in AD brains.

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Poster

696. Genetics and Epigenetics of Alzheimer's Disease and Related Models

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG014449

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Miles for Memories

Saint Mary's Foundation

Title: Correlation network analysis of frontal cortex gene expression changes during the progression of Alzheimer's disease

Authors: *S. E. COUNTS[1,2], E. MCKAY[1], Z. MADAJ[3], J. S. BECK[1], M. WINN[3]; [1]Transl Sci. and Mol Med, Family Med., Michigan State Univ., Grand Rapids, MI; [2]Hauenstein Neurosci. Ctr., St. Mary's Hosp., Grand Rapids, MI; [3]Bioinformatics and Biostatistics Core, Van Andel Inst., Grand Rapids, MI

Abstract: Alzheimer’s disease (AD) is characterized by the progressive dysregulation of multiple gene families in forebrain projection systems regulating cognitive function. However, the spatiotemporal overlap of these gene expression changes presents a challenge for pinpointing causal vs. epiphenomenal events. In this regard, gene expression profiling in frontal cortex during AD progression is an attractive option given the relatively delayed involvement of this region in AD pathogenesis and its ability to respond to the onset of mild cognitive impairment (MCI) by neuronal reorganization. To address these possibilities, we performed microarray analysis using frozen frontal cortex (BA10) samples from Rush Religious Orders Study subjects who died with a clinical diagnosis of no cognitive impairment (NCI), MCI, or AD (n = 12/diagnostic group). Total RNA was analyzed using NimbleGen 12 x 135K arrays and probe intensity levels were quantified with RMA preprocessing (NimbleScan v2.5). A systems-based Weighted Gene Co-expression Network Analysis (WGCNA) was then performed to identify modules of co-expressed genes that correlated with disease progression based on clinical or neuropathological diagnostic criteria. For example, a network module annotated by clinical diagnosis was enriched for insulin signaling (FDR p = 0.016) and included hub genes associated with cell cycle control, including p16INK4, and p14ARF. By contrast, a network module annotated by Braak and CERAD diagnosis was enriched for hypoxia signaling (FDR p = 0.04) and included hub genes associated with cell surface adhesion, including the integrin ITGA6 and

the α-secretase ADAM10. Intriguingly, Braak stage variability among the cases was most strongly associated with peroxisomal alkylglycerone phosphate synthase (AGPS) expression (FDR p = 0.007). Additionally, second order linear modeling was performed to identify eigengene networks correlated with differential expression in the MCI diagnostic group. Notably, annotation by clinical diagnosis identified the hub gene FOXP2, a transcription factor prominently involved in language development, whereas annotation by CERAD score variability was associated with the natural killer cells KLRC2 (FDR p = 0.002) and KLRC3 (FDR p = 0.0007). These gene networks may represent underlying plasticity responses in frontal cortex associated with the onset of dementia. Taken together, these data suggest that multiple gene co-expression network alterations in frontal cortex may provide new clues to the biological systems underlying the onset and progression of AD.

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Poster

696. Genetics and Epigenetics of Alzheimer's Disease and Related Models

Location: Halls B-H

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: P01AG14449

R01AG04375

P01AG017617

Title: Gene expression profiling of cathepsin D immunopositive pyramidal neurons within the precuneus in mild cognitive impairment and Alzheimer’s disease

Authors: *B. HE[1], S. E. PEREZ[1], S. LEE[2], E. PETRKOVA[3], S. D. GINSBERG[2], E. J. MUFSON[1];
[1]Neurobio., Barrow Neurolog. Inst., Phoenix, AZ; [2]Dept. of Psychiatry, Nathan Kline Inst., Orangeburg, NY; [3]Child and Adolescent Psychiatry, New York Univ. Langone Med. Ctr., New York, NY

Abstract: The precuneus is a component of the default memory network (DMN). Antemortem amyloid imaging studies using Pittsburgh compound B (PiB) revealed that this cortical region is particularly vulnerable to amyloid-beta (Aβ) deposition in the earliest, pre-clinical stages of Alzheimer’s disease (AD), suggesting that Aβ deposition plays a role in the neuronal dysfunction

of this component of the DMN in AD. However, there is virtually no information on the effect that amyloid deposition has upon gene expression of presumed vulnerable neurons in the precuneus. Numerous studies indicate the endosomal-lysosomal (E-L) pathway plays a key role in amyloid-beta precursor protein (APP) processing and Aβ generation. Although E-L activation occurs prior to the appearance of Aβ pathology, the effect of amyloid pathology upon gene expression in precuneus neurons displaying lysosomal network activation remains to be determined. Cathepsin D (Cat D), the main acid hydrolase in human lysosomes, is over-expressed early in vulnerable cortical neurons and is implicated in the pathogenesis of AD. Here, we employed Cat D immunohistochemistry to identify layer III pyramidal neurons from the precuneus and interrogated them via laser capture microdissection coupled with custom-designed microarray analysis to determine their genetic signature from cases that came to autopsy with a preclinical diagnosis of no cognitive impairment (NCI), mild cognitive impairment (MCI) and AD from the Rush Religious Orders Study. Preliminary results indicate approximately 15% of the transcripts on the array platform were differentially regulated between AD and NCI, 8% between MCI and NCI, and 17% between AD and MCI, indicating both early and late gene expression level changes during the progression of dementia. Classes of transcripts that displayed significant levels of alterations included cytoskeletal elements, cholinergic and monoaminergic neurotransmission markers, and cell death genes. Individual genes with notable expression differences include significant upregulation of the cholinergic synthesis regulatory elements choline acetyltransferase (ChAT), acetylcholinesterase (AChE), and butyrylcholinesterase (BChE) in AD compared to NCI and MCI. These findings suggest compensatory upregulation of the cholinergic system as part of a presumed neuroplasticity response, consistent with alterations observed in the frontal cortex and hippocampus at the early stages of AD pathology. Furthermore, the profiling of precuneus neurons did not reveal alterations in Aβ transcripts suggesting a disconnection between elevated Aβ binding and the upregulation of genes within this DMN region.

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Poster

696. Genetics and Epigenetics of Alzheimer's Disease and Related Models

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Program#/Poster#: 696.09/R17

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: AG043375

AG017617

AG014449

Title: Single population high throughput transcriptomic analysis of hippocampal and neocortical pyramidal neurons in mouse and postmortem human brain

Authors: *S. D. GINSBERG[1,2,3], A. SALTZMAN[1], I. DOLGALEV[4], A. HEGUY[4,5], M. J. ALLDRED[1,2];
[1]Ctr. for Dementia Res., Nathan S Kline Inst., Orangeburg, NY; [2]Psychiatry, [3]Neurosci. & Physiol., [4]Genome Technol. Ctr., [5]Pathology, New York Univ. Langone Med. Ctr., New York, NY

Abstract: High-throughput functional genomics enables gene transcripts and noncoding RNAs (ncRNAs) to be assessed quantitatively, which is relevant towards understanding the molecular pathogenesis of Alzheimer's disease (AD). RNA sequencing (RNA-seq) is at the center of this paradigm shift. To date, the majority of RNA-seq studies in AD and AD models are at the regional level. However, performing RNA-seq using RNA extracted from homogeneous populations of cells is possible. This approach avoids contamination from relatively spared neuronal and non-neuronal cells. To test the feasibility of single population RNA-seq, CA1 pyramidal neurons and layer II/III cortical pyramidal neurons were microaspirated from 20 μm thick frozen tissue sections mounted onto PEN membrane slides (Leica) obtained from C57Bl/6 mice and nondemented human brains by laser capture microdissection (LCM; Leica). Tissue sections were rapidly stained with Nissl and ~500 cells were acquired per reaction, per cell type. CA1 pyramidal neurons and neocortical pyramidal neurons were isolated and RNA was extracted. Preliminary data using LCM-captured cortical pyramidal neurons from wild-type mice and postmortem nondemented human brain indicate that 500 neurons were sufficient to generate robust cDNA libraries for RNA-seq. High quality RNA was demonstrated with clear 18S and 28S peaks by bioanalysis and RIN values of 4-6 after LCM. cDNAs from murine and human cortical pyramidal neurons were of high concentration and fragmented to sizes between 300-500 basepairs per the RNA-seq protocol. RNA sequencing libraries were generated in conjunction with the HiSeq 2500 platform (Illumina). Sequencing results were de-multiplexed and converted to FASTQ format using Bcl2FastQ software (Illumina). Paired-end reads were aligned to the mouse and human genomes (build mm10/ GRCm38 and hg19/GRCh37, respectively) using the splice-aware STAR aligner. HTSeq was employed to generate counts for each gene based on how many aligned reads overlap exon sequences. Counts were normalized and used to test for differential expression using negative binomial generalized linear models implemented by the DESeq2 R. By employing RNA-seq, we will be able to examine changes seen in the entire transcriptome of select vulnerable neuronal populations, which will be invaluable for determining mechanisms underlying neurodegenerative disorders without contamination of admixed cell types.

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Poster

696. Genetics and Epigenetics of Alzheimer's Disease and Related Models

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Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Sex differences in the molecular pathology of the 5XFAD mouse model of Alzheimer's disease

Authors: *J. L. BUNDY[1], C. VIED[2], C.-D. BADGER[1], R. S. NOWAKOWSKI[1];
[1]Biomed. Sci., [2]Translational Sci. Lab., Florida State Univ. Col. of Med., Tallahassee, FL

Abstract: Alzheimer's Disease (AD), a progressive neurodegenerative disorder, is the most common form of dementia, affecting approximately 48 million people worldwide. AD has been shown to have a sex-biased epidemiological profile, affecting twice as many women as men. In an effort to elucidate molecular mechanisms that provide a basis for this difference, we conducted a molecular investigation of both females and males using the 5XFAD mouse model of AD. This investigation focused on early stages of disease development (1, 2, and 4 months of age) using RNA-sequencing of the hippocampus of heterozygous 5XFAD males and females. At one month of age, only the transgene is differentially expressed. At 2 months of age, some molecular pathological changes could be detected with 39 transcripts identifiable as differentially expressed between transgenic animals and wild-type littermates. By 4 months of age, disease-associated transcriptomic changes are wide-spread, with over 1300 differentially expressed transcripts identified. Pairwise comparisons between 4 month old transgenic females and males indicate that disease-associated genes are more highly-expressed in female 5XFAD mice than their male counterparts. Furthermore, comparisons of female transgenic vs female wild-type animals yield more differentially expressed genes than the comparison between transgenic and wild-type males, reinforcing the finding that disease-associated molecular changes are more wide-spread in female animals. Overall, these data indicate that female 5XFAD mice exhibit more profound AD-like molecular pathology than their male counterparts. The sex-biased genes identified in this investigation are possible contributors to the observed sex-bias in AD in humans, and also provide potential candidate targets for pharmacologic intervention for disease amelioration or prevention.

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Poster

696. Genetics and Epigenetics of Alzheimer's Disease and Related Models

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant RO1-AG031517

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Title: Apoeε4 status impacts exercise-induced serum bdnf levels in elderly african americans with mci.

Authors: *J. S. ALLARD[1,2,3], O. NTEKIM[2], S. P. JOHNSON[3], J. S. NGWA[4], R. F. GILLUM[5], T. V. FUNGWE[2], T. O. OBISESAN[3];
[1]Physiol. and Biophysics, Howard Univ. Col. of Med., Washington, DC; [2]Dept. of Nutr., Howard Univ. Sch. of Nursing and Allied Hlth. Sci., Washington, DC; [3]Geriactrics Division, Dept. of Med., [4]Cardiovasc. Med., [5]Dept. of Med., Howard Univ. Hosp., Washington, DC

Abstract: Possession of the Apolipoprotein E (ApoE) gene ε4 polymorphism is the most prevalent genetic risk factor for Alzheimer's disease (AD), and is also associated with other neurodegenerative conditions. Evidence indicates that ApoE plays important roles in critical brain functions including response to brain injury, cerebrovascular function and synaptic plasticity. Recent evidence has also suggested that ApoE genotype differentially affects expression of brain-derived neurotrophic factor (BDNF). Notably, aerobic exercise-induced upregulation of BDNF is well documented; and exercise has been shown to improve cognitive function, in many disease states, in both young and elderly populations. Thus, BDNF upregulation is a highly proposed mechanism for the cognitive-enhancement effects of physical exercise. In this study we examined the effects of ApoEε4 carrier status on exercise-induced changes in BDNF expression. Elderly African Americans diagnosed with mild cognitive impairment participated in a six-month, supervised program of either stretch or aerobic exercise. Participants engaged in 40 minutes of supervised exercise training 3 times per week. Aerobic exercise consisted of treadmill walking or jogging at an initial intensity that correlated with 50% VO_2Max and gradually increased to 70% VO_2Max. Stretch exercise consisted of a variety of dynamic stretch positions that were held for 15-30 second intervals. BDNF levels in serum were measured using ELISA. Age, BMI, MMSE scores, VO_2Max and baseline BDNF did not differ between ApoEε4 carriers and non-ε4 carriers. There was a statistically significant interaction between ApoEε4 status and 6-month serum BDNF levels ($p=0.010$). Non-ε4 carriers showed a significant increase in BDNF levels at the 6 month exercise time point, while ε4 carriers showed no significant effect of the exercise intervention. We have identified an important relationship

between the ApoEε4 genetic polymorphism and BDNF response to physical activity which likely impacts the extent of neuroprotective benefit gained from engagement in physical exercise.

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Poster

696. Genetics and Epigenetics of Alzheimer's Disease and Related Models

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NWO grant 022.005.019

JPND project 733051064

Title: Alzheimer's disease-associated genome-wide DNA methylation and hydroxymethylation patterns in the middle temporal gyrus

Authors: ***R. LARDENOIJE**[1], J. ROUBROEKS[1,2], E. PISHVA[1], A. IATROU[1], B. RUTTEN[1], G. KENIS[1], H. W. M. STEINBUSCH[1], D. MASTROENI[3], P. COLEMAN[3], E. DELVAUX[3], A. SMITH[2], R. SMITH[2], K. LUNNON[2], D. L. A. VAN DEN HOVE[1,4];
[1]Maastricht Univ., Maastricht, Netherlands; [2]Univ. of Exeter, Devon, United Kingdom; [3]Arizona State Univ., Tempe, AZ; [4]Univ. of Würzburg, Würzburg, Germany

Abstract: Over last few years, ample evidence has been generated pointing towards an epigenetic dysregulation in Alzheimer's disease (AD). Our understanding of the full scope of the epigenetic apparatus is still rapidly evolving, with techniques to investigate the mechanisms involved barely keeping up. DNA methylation is one of the most studied epigenetic mechanisms, and many of the methods used depend on bisulfite (BS) conversion of the DNA to distinguish methylated from non-methylated cytosines. The inability of these methods to distinguish DNA methylation from DNA hydroxymethylation, another, functionally different epigenetic modification, has been somewhat neglected due to methylated cytosines generally being much more abundant. This limitation, however, may be especially problematic in brain tissue, which is enriched in hydroxymethylated cytosines. As some of the major findings in relation to methylomic alterations in relation to AD have been made using Illumina's Infinium HumanMethylation450 BeadChip (450k) array, we used a recently established technique using both BS converted and oxidative bisulfite (oxBS) converted DNA, to investigate the methylome and hydroxymethylome in the middle temporal gyrus of 47 AD patients and 36 controls.

Differentially (hydroxy)methylated positions (D(h)MPs) and regions (DMRs) related to AD were determined and compared with those resulting from the combined methylation and hydroxymethylation signal. Previously reported genes with altered DNA methylation in AD, *ANK1* and *RHBDF2*, were in our top 100 for the combined signal and the methylation signal only, indicating these genes are only affected by alterations in DNA methylation in AD. Interestingly, we identify a DMP and DMR, and a DhMP in the *OXT* gene, suggesting this gene is regulated through both methylation and hydroxymethylation. Genes associated with our top DMPs, include *SYNJ2*, *PRKCSH* and *EFNA3*, and genes associated with our top DhMPs, include *GNB3*, *PDE4D* and *PRKCSH*. We also investigated the relation between DNA (hydroxyl)methylation and mRNA expression. In conclusion, as there are currently no arrays available for the genome-wide investigation of DNA hydroxymethylation, we used an approach based on the 450k array, combining BS and oxBS treated DNA, and identified genes with altered methylation or hydroxymethylation in AD.

Disclosures: **R. Lardenoije:** None. **J. Roubroeks:** None. **E. Pishva:** None. **A. Iatrou:** None. **B. Rutten:** None. **G. Kenis:** None. **H.W.M. Steinbusch:** None. **D. Mastroeni:** None. **P. Coleman:** None. **E. Delvaux:** None. **A. Smith:** None. **R. Smith:** None. **K. Lunnon:** None. **D.L.A. van den Hove:** None.

Poster

696. Genetics and Epigenetics of Alzheimer's Disease and Related Models

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 696.13/S4

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Genetic determinants of clinical progression in Alzheimer's disease: a pilot study

Authors: *B. A. KENT, L. TOOMAN, I. GUELLA, D. EVANS, E. DWOSH, D. SADOVNICK, G.-Y. HSIUNG, M. J. FARRER, H. B. NYGAARD;
Djavad Mowafaghian Ctr. for Brain Hlth., Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Recent Genome-wide Association Studies (GWAS) and the wider availability of High Throughput Sequencing (HTS) continue to refine our understanding of the genetic basis for Alzheimer’s disease. To date, more than 22 genes that increase the risk of AD have been identified, providing targets for potential therapeutic intervention. However, the genetic basis for clinical progression in AD is not well understood. To identify deterministic genetic factors for disease progression in AD, we collected 100 multi-incident families. All patients were followed in the UBC Hospital Clinic for Alzheimer disease and Related Disorders, with deep, longitudinal cognitive assessments. Rate of cognitive decline up to 10 years from symptom onset was

recorded by measuring standardized scores on the Montreal Cognitive Assessment Scale (MoCA), Mini-Mental State Examination (MMSE), and the Modified Mini Mental (3MS) exams. Whole exome analysis was performed on 100 affected probands using 57Mb Ampliseq™ library preparation with an Ion Proton system. Results were complemented by Illumina MEGA-Ex genotyping (1.8M single nucleotide polymorphisms (SNPs)) and subsequent copy number analyses. All known and novel variants in genes linked/associated with dementia syndromes were documented; when required pathogenicity was confirmed by Sanger sequencing or Taqman probes, and segregation analyses within families. Association between SNPs and long-term clinical disease progression was assessed using logistic regression models. A genetic panel to predict clinical progression would be a valuable tool in the overall assessment of patients with AD.

Disclosures: **B.A. Kent:** None. **L. Tooman:** None. **I. Guella:** None. **D. Evans:** None. **E. Dwosh:** None. **D. Sadovnick:** None. **G. Hsiung:** None. **M.J. Farrer:** None. **H.B. Nygaard:** None.

Poster

696. Genetics and Epigenetics of Alzheimer's Disease and Related Models

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 696.14/S5

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Training Grant 5T32AG000216-24

NIH Pilot Award UL1 TR001114-03

Shaffer Family Foundation

Title: Down Syndrome and Alzheimer's Disease brains contain neurons with increased genomic mosaicism

Authors: ***G. E. KAESER**, B. SIDDOWAY, M.-H. LEE, S. ROHRBACK, C. SAUVEY, J. CHUN;
The Scripps Res. Inst., La Jolla, CA

Abstract: Traditional understanding of the genome is that every cell in the human body contains a constant and identical genomic sequence. However, a growing body of evidence has shown that neurons in the brain are an exception to this dogma. Neurons within a single brain display genomic mosaicism, the phenomenon that is produced by neurons having distinct genomes, like individual tiles in a mosaic. Genomic mosaicism encompasses aneuploidy, copy number

variation, line-1 elements, and single nucleotide variants. We first identified brain aneuploidies in neural progenitors and functionally integrated neurons; and documented region-specific changes in total DNA content in human neurons by DNA flow cytometry. Recently, we identified an ~8% increase in total DNA content in sporadic Alzheimer's disease (SAD) prefrontal cortical neurons compared to controls, that was accompanied by mosaic *APP* copy number amplifications, and independent of trisomy 21 [Bushman et al., *eLife* 2015]. This work demonstrated the first functional significance for mosaic DNA changes in neurons. We assessed the possibility that genomic mosaicism may also function in other neurodegenerative diseases, including genetic forms of AD via the well-documented AD association with Down Syndrome (DS). Remarkably, DS cortices, from age 0-60 years, displayed a highly significant linear increase in total DNA content that was not observed in non-diseased brains. The greatest increases were observed in brains displaying AD pathology, and increases were equivalent to those observed in SAD brains. These data implicate genomic mosaicism and DNA content increases as common events in both SAD and genetic AD. Importantly, the linearity of increasing DNA implicates post-mitotic neuronal DNA synthesis accumulating with age. Targeted genomic analyses of cell populations and single cells are under investigation and preliminary data have revealed mosaic copy number events and unique structural alterations.

Disclosures: G.E. Kaeser: None. **B. Siddoway:** None. **M. Lee:** None. **S. Rohrback:** None. **C. Sauvey:** None. **J. Chun:** None.

Poster

696. Genetics and Epigenetics of Alzheimer's Disease and Related Models

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 696.15/S6

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Role of Unc5c, Alzheimer's risk gene in late-onset Alzheimer's disease

Authors: *D. KARUNAKARAN[1], J. ATWAL[2], R. VASSAR[1];
[1]Northwestern Univ., Chicago, IL; [2]Genentech, South San Francisco, CA

Abstract: Alzheimer's disease (AD) is characterized by amyloid plaques, neurofibrillary tangles, and synaptic and neuronal loss. Recently, a rare autosomal dominant coding mutation, T835M, was discovered in the Un-coordinated 5c (Unc5c) netrin receptor gene that segregated with late-onset AD (LOAD). T835M alters a conserved amino acid in the hinge region of the Unc5c death domain, suggesting the mutation may increase apoptosis. Indeed, in primary hippocampal neurons, overexpression of Unc5c T835M increased cell death in response to neurotoxic stimuli including beta-amyloid (Aβ). These results suggest a mechanism by which Unc5c T835M may

confer increased risk of LOAD, however the effects of this mutation in an AD animal model have not yet been explored. Toward this end, we have obtained a mouse knock in (KI) model of Unc5c T85M that we will cross with the 5XFAD mouse model of amyloid pathology and neuron loss. We hypothesize that the Unc5c T835M mutation will exacerbate neuronal death in the 5XFAD brain via increased sensitivity to Aβ-induced neurotoxicity and Unc5c death domain activation. We will investigate mechanisms of cell death in 5XFAD; Unc5c T835M KI mice by behavioral, biochemical, and cellular approaches, and unbiased RNA deep sequencing (RNAseq). Although neuron loss is a hallmark of AD, the exact molecular mechanism of cell death in AD is unclear. We expect our results to provide valuable insight into AD-associated cell death and how Unc5c is involved in AD pathogenesis, and thereby identify novel therapeutic targets for preventing neuron loss in AD.

Disclosures: D. Karunakaran: None. **J. Atwal:** None. **R. Vassar:** None.

Poster

696. Genetics and Epigenetics of Alzheimer's Disease and Related Models

Location: Halls B-H

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Program#/Poster#: 696.16/S7

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG034214

NIH Grant AG05136

Jane and Lee Seidman Fund

Title: FYN expression is associated with regulatory region genetic variation in Alzheimer's disease

Authors: *J. A. ZAHRATKA[1], Y. SHAO[1], M. SHAW[1], K. TODD[1], M. KHRESTIAN[1], T. MONTINE[2], J. B. LEVERENZ[3], L. M. BEKRIS[1];
[1]Genomic Med. Inst., Cleveland Clin. Lerner Res. Inst., Cleveland, OH; [2]Pathology, Stanford Univ., Stanford, CA; [3]Lou Ruvo Ctr. for Brain Hlth., Cleveland Clin., Cleveland, OH

Abstract: Alzheimer’s disease is characterized by neuritic plaques, consisting primarily of amyloid-beta (Aβ) aggregates, and neurofibrillary tangles (NFTs), assemblies of hyperphosphorylated forms of the protein tau. Tau phosphorylation is under the control of multiple kinases and phosphatases, including GSK3β, Fyn, and PP2A. Previously, our group correlated a regulatory region single nucleotide polymorphism (SNP), rs7768046, in the *FYN* gene as associated with increased cerebrospinal fluid total tau levels. In this study, we

hypothesized that *FYN* expression in the brain is directly influenced by AD status and genetic content. Our results suggest that *FYN* mRNA levels stay constant but protein levels are increased in AD patients, which are further altered by regulatory SNPs in each *FYN* isoform promoter as well as the shared 3' untranslated region (3' UTR). Additionally, expression of the *FYN* 3' UTR can differentially inhibit *FYN* isoform promoter activity in multiple human cell lines, suggesting regulatory region variation can play an important role in *FYN* modulation and isoform expression. Further study supports a potential role for microRNAs (miRNAs) participating in regulatory roles of *FYN* expression. Several miRNA are predicted to target the *FYN* 3'UTR. Of these predicted miRNA a few are differentially expressed in AD compared to control post-mortem hippocampus. Our preliminary data suggest two of these differentially expressed miRNAs, hsa-miR-200c-3p and hsa-miR-15a-5p, do not significantly affect *FYN* expression in CHP-212 or SH-SY5Y cells. Taken together, these data suggest that *FYN* expression is regulated according to AD status and promoter haplotype, and such genetic variants may be instrumental in the development of NFTs in AD and other tauopathies.

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Poster

696. Genetics and Epigenetics of Alzheimer's Disease and Related Models

Location: Halls B-H

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Program#/Poster#: 696.17/S8

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH UL1 TR001114-03 Pilot Award

The Shaffer Family Foundation

Title: Mosaic genomic variation in the sporadic Alzheimer's disease brain

Authors: ***C. SAUVEY**[1], M.-H. LEE[2], G. KAESER[2], B. SIDDOWAY[2], J. CHUN[2];
[1]The Scripps Res. Inst., LA Jolla, CA; [2]The Scripps Res. Inst., La Jolla, CA

Abstract: Alzheimer's Disease (AD) is a common, devastating, progressive neurodegenerative disease that results in dementia and eventually patient death. While a wide variety of theories exist regarding AD's underlying pathological mechanism, the consensus agrees on the involvement of the proteins amyloid β (Aβ) and Tau, encoded by the APP and MAPT genes respectively. Aβ is a major component of the amyloid plaques, and Tau forms the neurofibrillary tangles, which are both suspected of playing a causative role in the development or pathogenesis

of AD. While modern biology holds that nearly all healthy cells in a given organism are genomically identical, several reports published within the past decade have begun to call this assumption into question. These reports found that in both developing and mature mammalian brains many different types of large-scale genomic alterations such as aneuploidy and copy number variations (CNVs) occur in a mosaic fashion. This phenomenon is referred to as genomic mosaicism. We recently reported neuronal genomic mosaicism in the form of increased DNA content variation (DCV) associated with sporadic AD. (Bushman et al., *eLife* 2015). Based upon these findings, we have pursued further characterization of DNA content changes using flow cytometry and fluorescence activated cell sorting (FACS) analyses of isolated NeuN+ nuclei. Analyses have focused on variations associated with 1) SAD and other neurodegenerative disorders, 2) brain regions, and 3) neuronal subtypes. Preliminary data indicate that non-random alterations to DCV are occurring regionally and in neuronal subtypes, and current efforts are identifying changes in putative genes associated with DCV. These findings have implications for our understanding of both origins and roles for genomic mosaicism in SAD.

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Poster

696. Genetics and Epigenetics of Alzheimer's Disease and Related Models

Location: Halls B-H

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Topic: C.02. Alzheimer's Disease and Other Dementias

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Hong Kong Research Grants Council Theme-based Research Scheme T13-607/12R

Title: Fine mapping of the APOE locus by low-pass whole-genome sequencing in Chinese Alzheimer's disease patients

Authors: *X. ZHOU[1,2,3,4], Y. CHEN[1,2,3,4,5], Q. ZHAO[6], M.-K. M. CHU[1,2,3], C. T. KWOK[1,2,3], K. Y. MOK[1,2,3,7], Y. CHEN[1,2,3,4,5], B. ZHANG[8], A. K. FU[1,2,3,4], Y. LI[9], Q. GUO[6], N. Y. IP[1,2,3,4]; [1]Div. of Life Sci., [2]Mol. Neurosci. Ctr., [3]State Key Lab. of Mol. Neurosci., The Hong Kong Univ. of Sci. and Technol., Clear Water Bay, Hong Kong, China; [4]Guangdong Key Lab. of Brain Science, Dis. and Drug Develop., HKUST Shenzhen Res. Inst., Shenzhen, Guangdong, China; [5]The Brain Cognition and Brain Dis. Institute, Shenzhen Inst. of Advanced Technol., Chinese Acad. of Sci., Shenzhen, China; [6]Dept. of Neurol. and Inst. of Neurology, Huashan Hosp., Fudan Univ., Shanghai, China; [7]Dept. of Mol. Neurosci., UCL Inst. of Neurol., London, United Kingdom; [8]Dept. of Neurology, The Second Affiliated Hospital, Sch. of Med., Zhejiang Univ., Hangzhou, Zhejiang, China; [9]Dept. of Genet., Univ. of North Carolina at Chapel Hill, Chapel Hill, NC

Abstract: Alzheimer's disease (AD) is an age-related neurodegenerative disease accounting for 60-70% of dementia cases. Large-scale genetic studies have aimed to elucidate potential risk genes. Among the approximately 300 genes studied in different AD cohorts to date, APOE has emerged as the most consistent marker associated with late-onset AD, the most common form of AD. Although the allele frequency of APOE is moderately lower in the Chinese population than Caucasian populations, few genetic studies of the Chinese AD population have been conducted. Here, we analyzed the APOE locus using our in-house low-pass whole-genome sequencing data from a Chinese AD cohort. We not only confirmed the existence of a long-range haplotype in the 40-kb region around APOE, which is strongly associated with AD, but also identified other novel variants linked to AD in this region. These results suggest a multigenic effect contributing to the late-onset AD pathogenesis in chromosome 19q13.32 involving PVRL2, TOMM40, APOE, and APOC1. Our findings will facilitate the determination of potential biomarkers for the diagnosis of AD in the Chinese population.

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Poster

696. Genetics and Epigenetics of Alzheimer's Disease and Related Models

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Postdoctoral Research Abroad Program Fellowship from Ministry of Science and Technology, Taiwan/105-2917-I-564-085

NIH Pilot Award/UL1 TR001114-03

NIH Training Grant/5T32AG000216-24

Staffer Family Foundation

Title: New elements of genomic mosaicism in sporadic Alzheimer's disease

Authors: *M.-H. LEE, G. E. KAESER, R. R. RIVERA, B. SIDDOWAY, C. R. SAUVEY, S. E. ROHRBACK, W. S. MCDONALD, Y. WEI, J. CHUN;
DEPARTMENT OF MOLECULAR AND CELLULAR NEUROSCIENCE, THE SCRIPPS RESEARCH INSTITUTE, San Diego, CA

Abstract: Alzheimer’s disease (AD) is a devastating neurodegenerative disease and clinically characterized by cognitive function decline, short-term memory loss, language disturbance, and executive function impairment. A relatively new feature of the normal brain is genomic mosaicism, that consists of aneuploidy, LINE elements, copy number variations, and single nucleotide variations. Recently, genomic mosaicism was identified in sporadic AD (SAD) brains and was associated with increased DNA content and APP copy number increases, supporting the operation of genomic mosaicism mechanisms in SAD (Bushman *et al*. eLIFE, 2015). To further explore genomic mosaicism in SAD, small populations of neuronal nuclei were stained by propidium iodide and NeuN, and isolated by fluorescence-activated cell sorting (FACS). Genomic DNA was extracted and being assessed by a range of PCR strategies including multiplex methodologies, using collections of primers designed against a range of AD-related genes - both coding and non-coding regions - including those identified from genome-wide association studies (GWAS). Preliminary results have identified regionally distinct signals that are consistent with genomic mosaicism, and further validation is ongoing, results of which will be presented.

Disclosures: M. Lee: None. **G.E. Kaeser:** None. **R.R. Rivera:** None. **B. Siddoway:** None. **C.R. Sauvey:** None. **S.E. Rohrback:** None. **W.S. McDonald:** None. **Y. Wei:** None. **J. Chun:** None.

Poster

696. Genetics and Epigenetics of Alzheimer's Disease and Related Models

Location: Halls B-H

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NLM R00 LM011384-02

Title: Interaction of NMDA-receptor antagonist medication use and ADORA2A genotype on hippocampal volume in patients with Mild Cognitive Impairment and Alzheimer's Disease

Authors: *E. HORGUSLUOGLU-MOLOCH[1,2], K. NHO[3,4], S. L. RISACHER[3,2], A. J. SAYKIN[3,2];
[1]Med. and Mol. Genet., [2]Indiana Alzheimer Dis. Ctr., [3]Radiology and Imaging Sci., [4]Computat. Biol. and Bioinformatics, Indiana University, Sch. of Med., Indianapolis, IN

Abstract: Alzheimer's disease (AD), the most common form of dementia, includes the features of decline in memory impairment and other cognitive domains as well as prominent hippocampal atrophy. Although there is no disease modifying medication for AD yet, cholinesterase inhibitors and an NMDA (N-methyl-D-aspartate) receptor antagonist (memantine HCL) are often used clinically. Previously, we performed a gene-based association analysis of neurogenesis pathway-related candidate genes in participants from the Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort and identified *ADORA2A* and a SNP (rs9608282-T) upstream of *ADORA2A* to be significantly associated with larger hippocampal volume and better memory scores. Based on previous studies suggesting that *ADORA2A* plays an important role controlling NMDA-dependent synaptic toxicity and memory impairment (Tebano, Martire et al. 2005, Yee, Singer et al. 2007, Sarantis, Tsiamaki et al. 2015), we examined the interaction of memantine use and rs9608282 (*ADORA2A*) on hippocampal volume and memory performance. **Methods:** Non-Hispanic Caucasian participants (N=1,102) from ADNI with structural magnetic resonance imaging (MRI) scans were included. We evaluated the effect of rs9608282 on hippocampal volume and composite memory score in ADNI participants diagnosed with MCI (mild cognitive impairment) or AD. Participants were further classified as a memantine user (NMDA (+)) or a memantine non-user (NMDA (-)). Two-way analysis of covariance (ANCOVA) for continuous variables and chi-square for categorical variables were performed in SPSS 23.0. **Results:** NMDA (-) participants carrying at least one copy of the minor allele (T) of *ADORA2A* rs9608282 had a larger mean hippocampal volume ($p<0.001$). There was also a significant interaction of NMDA-receptor antagonist use and *ADORA2A* rs9608282 on memory performance ($p = 0.009$). NMDA (+) participants carrying at least one copy of the minor allele (T) of the *ADORA2A* rs9608282 had poorer memory performance. **Conclusion:** The synaptic localization of A2a receptors (ADORA2A) plays a key role controlling NMDA-dependent synaptic transmission in the hippocampus (Rebola, Sachidhanandam et al. 2007). Individuals with genetic variation in *ADORA2A* gene associated with glutamate signaling pathway may show differential responsiveness to NMDA-receptor targeted treatment.

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Poster

696. Genetics and Epigenetics of Alzheimer's Disease and Related Models

Location: Halls B-H

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Topic: C.02. Alzheimer's Disease and Other Dementias

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Title: Cholinergic surveillance over hippocampal RNA metabolism and Alzheimer's-like pathology

Authors: *M. A. PRADO[1], B. KOLISNYK[2], M. AL-ONAIZI1[2], L. SOREQ[3], J. ULE[3], H. SOREQ[4], V. PRADO[2];
[1]Robarts Res. Institute/University of Western O, London, ON, Canada; [2]Robarts Res. Institute/University of Western Ontario, London, ON, Canada; [3]Dept. of Mol. Neurosci., UCL Inst. of Neurol., London, United Kingdom; [4]The Edmond and Lily Safra Ctr. for Brain Sci., The Hebrew Univ. of Jerusalem, Jerusalem, Israel

Abstract: The relationship between long-term cholinergic dysfunction and risk of developing dementia is poorly understood. Here we used mice with deletion of the vesicular acetylcholine transporter (VAChT) in the forebrain to model cholinergic abnormalities observed in dementia. Whole genome RNA-sequencing of hippocampal samples revealed that cholinergic failure causes changes in RNA metabolism. Remarkably, key transcripts related to Alzheimer's disease are affected. *BACE1* for instance, shows abnormal splicing caused by decreased expression of the splicing regulator hnRNPA2/B1. Resulting BACE1 overexpression leads to increased APP processing and accumulation of soluble $A\beta_{1\text{-}42}$. This is accompanied by age-related increases in GSK3 activation, tau hyper-phosphorylation, caspase-3 activation, decreased synaptic markers, increased neuronal death and deteriorating cognition. Pharmacological inhibition of GSK3 hyperactivation reversed deficits in synaptic markers and tau hyperphosphorylation induced by cholinergic dysfunction, indicating a key role for GSK3 in some of these pathological changes.

These results suggest that changes in RNA processing caused by cholinergic loss can facilitate Alzheimer's-like pathology in mice, providing a mechanism by which decreased cholinergic tone may increase risk of dementia.

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Poster

696. Genetics and Epigenetics of Alzheimer's Disease and Related Models

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Topic: C.02. Alzheimer's Disease and Other Dementias

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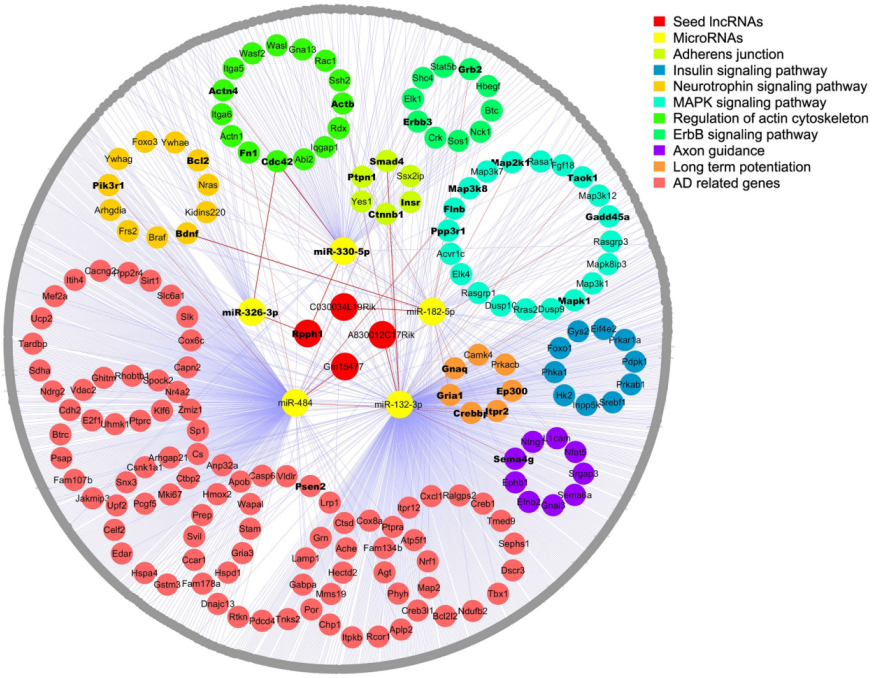
Municipality for Peacock Plan Research Grant No.KQC201105300001A

Title: Rpph1 regulates Cdc42 by competing endogenous miR-330-5p and modulates dendritic spine formation in hippocampal pyramidal neurons

Authors: ***Y. CAI**[1,2], Z. SUN[2], H. LUO[2], Q. WU[2], J. WAN[2,3];
[1]SZ-PKU-HKUST Med. Ctr., Peking Univ., Beijing, China; [2]Shenzhen PKU-HKUST Med. Ctr., Shenzhen, China; [3]Hong Kong Univ. of Sci. and Technol., Hong Kong, China

Abstract: Background: Alzheimer's disease (AD) is a heterogeneous neurodegenerative disease, which accompanies with impaired synapses and memory loss. Compensations were reported in AD brains by enlarging the remaining synapses connections or by paraterminal sprouting of dendritic spines. However, the underlying mechanism is not yet understood. Recent studies showed that non-coding RNAs can crosstalk through microRNA response elements (MRE) and manipulate biological processes, but few was studied in AD. Working on the hypothesis that non-coding RNAs can mutually interact in a competing endogenous RNAs (ceRNA) manner and contribute to the AD processes, we attempted to identify the functional ceRNAs in AD and the biological processes that they involved.
Materials and methods: CeRNA network was constructed based on a whole transcriptome

sequencing and a previous studied microRNA-seq of APP/PS1 transgenic mice. CeRNA mechanism was testified by dual luciferase reporter assay. Overexpression and RNA interference assay was detected by quantitative RT-PCR and western blot in Neuro-2a cell line. Dendritic spine study was performed in primary cultured hippocampal neurons by calcium transfection. **Results:** A lncRNA-microRNA-mRNA network based on APP/PS1 mouse model was firstly demonstrated. Four seed lncRNAs and five hub miRNAs were mainly enriched in nine pathways and an AD related gene pool. Ribonuclease P RNA component H1 (Rpph1) is up-regulated APP/PS1 mice cortex compared to those of wild type and competes to bind miR-330-5p, subsequently increasing Cdc42 mRNA and protein level, moreover, overexpression of Rpph1 increased dendritic spine density in primary cultured hippocampal pyramidal neurons. Visa versa, knockdown of Rpph1 decreased Cdc42 expression level and dendritic spine density. **Conclusion:** These results indicate that Rpph1 post-transcriptionally regulates Cdc42 dynamics in a ceRNA manner, which can modulate dendritic spine formation in hippocampal neurons. Furthermore, these could be one of the molecular mechanisms underlying the compensations of AD process.



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Poster

697. Alzheimer's Disease: Imaging Techniques

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

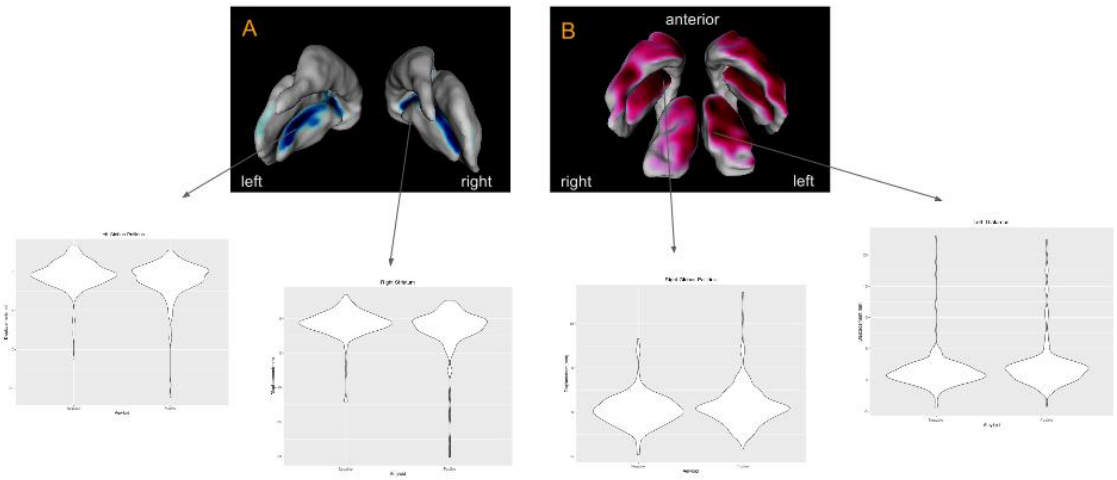
Program#/Poster#: 697.01/S14

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Influence of amyloid burden on subcortical volume and morphometry

Authors: *G. AYRANCI[1], R. PATEL[2], G. DEVENYI[1], V. KONG[3], M. CHAKRAVARTY[1,2,4], A. ALZHEIMER'S DISEASE NEUROIMAGING INITIATIVE[5];
[1]Cerebral Imaging Ctr. - Douglas Mental Hlth. Un, Verdun, QC, Canada; [2]Dept. of Biomed. Engin., McGill Univ., Montreal, QC, Canada; [3]McGill Univ., Integrated Program in Neuroscience, QC, Canada; [4]Dept. of Psychiatry, McGill Univ., Montreal, QC, Canada; [5]USC, Los Angeles, CA

Abstract: An essential hallmark of Alzheimer's Disease (AD) is β-amyloid plaque formation. However, a strong association between β-amyloid deposition and structural atrophy has been lacking. Thus, we sought to investigate the influence of amyloid burden on subcortical structures. Data obtained from Alzheimer's Disease Neuroimaging Initiative consisted of baseline 3T T1-weighted MR images and respective [18F]-florbetapir (FBP) PET analyses. Subjects were classified into amyloid positive or negative, using average FBP standardized uptake rate with cerebellum as a reference region, and AD patients excluded due to low power. MR images were pre-processed with minc-bpipe-library, and then processed with MAGeT brain for outputs of volume, vertex displacement and surface area. Influence of amyloid burden on subcortical volumes and morphometry were analyzed via multiple linear regression; accounting for diagnosis, age, sex, APOE ε4, and total brain volume. Analyses of vertex displacement and surface area were corrected for multiple comparisons with FDR.Our analyses did not show an effect of amyloid burden on the volume of either subcortical structure. Inward and outward pointing displacements in the amyloid positive individuals were observed in all structures analyzed in the absence of volume changes and survive correction for multiple comparisons at 1% false discovery rate and are highlighted in Fig 1. Our results point to a strong heterogeneity of amyloid burden, and suggest an overall effect on subcortical morphometry with no influence on subcortical volume, and provide further insight into the complex interplay between β-amyloid deposition and structural features in AD.



Linear modelling of amyloid burden on subcortical volume and morphometry: analyses did not show an effect of amyloid burden on the volume of either striatum (p = 0.88, left; p = 0.95, right hemispheres) or thalamus (p = 0.39, left; p = 0.81, right hemispheres), with a marginal decrease on the volume of right (p = 0.055) but not left (p = 0.13) globus pallidum (not shown). T-statistics mapped onto subcortical regions (corrected to 1%FDR) showed amyloid positive subjects had increased inward displacement in the external surface (A), counterbalanced with increased outward displacement (B) in the internal surface of globus pallidus. Same subjects also showed increased inward displacement in the precommissural caudate (A) with an outward displacement in the ventral posterior thalamus (B). Further exploration of peak vertices revealed strong heterogeneity of amyloid burden across individuals.

Disclosures: **G. Ayranci:** None. **R. Patel:** None. **G. Devenyi:** None. **V. Kong:** None. **M. Chakravarty:** None. **A. Alzheimer's Disease Neuroimaging Initiative:** None.

Poster

697. Alzheimer's Disease: Imaging Techniques

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 697.02/T1

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Department of Neurosurgery, Cedars-Sinai Medical Center

Donation of human brain tissue by Prof. H. V. Vinters, Department of Neurology, University of California, Los Angeles

Title: Polymalic acid-based targeted magnetic resonance imaging contrast agent for the detection of amyloid beta plaques in alzheimer’s disease

Authors: *L. A. MASHOUF[1,2], I. FOX[1], P. R. GANGALUM[1], S. WAGNER[1], H. DING[1], K. L. BLACK[1], J. Y. LJUBIMOVA[1], E. HOLLER[1], R. PATIL[1];
[1]Neurosurg., Cedars-Sinai Med. Ctr., Los Angeles, CA; [2]Neurosci., Johns Hopkins Univ., Baltimore, MD

Abstract: Alzheimer's disease (AD) is a gradual neurodegenerative disease that results in memory loss and can be accompanied by dementia and even death. Although AD is the 6th leading cause of death in the US, there remain few methods to detect its early onset. Currently, there is no gadolinium-based contrast agent available for conventional magnetic resonance imaging (MRI) detection of amyloidal beta (Aβ) plaques in Alzheimer's disease. Its timely finding would be vital for patient survival and quality of life. Curcumin (CUR), a common Indian spice, effectively binds to Aβ plaques, which is a hallmark of AD. To address this binding, we have designed a novel nanoimaging agent (NIA) based on nature-derived poly(β-L-malic acid) (PMLA) containing covalently attached gadolinium–DOTA(Gd–DOTA) and nature-derived CUR. The all-in-one agent recognizes and selectively binds to Aβ plaques and is detected by MRI. It efficiently detected Aβ plaques in human and mouse samples by an ex vivo staining. The method can be useful in clinic for safe and noninvasive diagnosis of AD.

Disclosures: L.A. Mashouf: None. **I. Fox:** None. **P.R. Gangalum:** None. **S. Wagner:** None. **H. Ding:** None. **K.L. Black:** None. **J.Y. Ljubimova:** None. **E. Holler:** None. **R. Patil:** None.

Poster

697. Alzheimer's Disease: Imaging Techniques

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 697.03/T2

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: GE Healthcare

Title: Relationship between ^{18}F-Flutemetamol uptake and RBANS performance in non-demented community-dwelling older adults

Authors: *T. ATKINSON[1], D. B. HAMMERS[1], B. C. ALLRED DALLEY[1], K. R. SUHRIE[1], B. BEARDMORE[2], L. BURRELL[2], K. RASMUSSEN[2], K. DUFF[1], J. M. HOFFMAN[2]; [1]Neurol., [2]Huntsman Cancer Inst., Univ. of Utah, Salt Lake City, UT

Abstract: The Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) has been used extensively clinically and for research on Mild Cognitive Impairment and Alzheimer's disease (AD), however relatively few studies have evaluated the relationship between RBANS performance and AD imaging biomarkers. The purpose of the current study was to evaluate the association between a relatively new amyloid imaging biomarker, ^{18}F-Flutemetamol, and performance on the RBANS and select behavioral measures in 27 non-demented, community-dwelling older adults. Amyloid deposition and RBANS Indexes of

Immediate Memory, Language, Attention, Delayed Memory, and Total Scale score were significantly correlated (p's < .05, d's = 0.91-2.16), with greater amyloid burden being associated with lower RBANS scores. The Delayed Memory Index was particularly highly associated with ^{18}F-Flutemetamol binding (r^2 = .53). Neither ^{18}F-Flutemetamol binding nor RBANS performance was significantly correlated with levels of depression or subjective report of cognitive difficulties. Because of the limited usage of amyloid-PET imaging in clinical settings due to high cost, these findings suggest that in particular RBANS Delayed Memory Index may be a cost-efficient tool to identify early signs of AD pathology, and its use may enlighten clinical decision making regarding potential progression to dementia due to AD.

Disclosures: T. Atkinson: None. **D.B. Hammers:** None. **B.C. Allred Dalley:** None. **K.R. Suhrie:** None. **B. Beardmore:** None. **L. Burrell:** None. **K. Rasmussen:** None. **K. Duff:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; Research Funding from GE Healthcare. **J.M. Hoffman:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; Research Funding from GE Healthcare.

Poster

697. Alzheimer's Disease: Imaging Techniques

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 697.04/T3

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH/NIA 1 R21 AG037843

Brigham Young University, College of Life Sciences, Start-Up Grant

Brigham Young University, School of Family Life, Gerontology Program

Dr. Sarah M. McGinty Neuroscience Graduate Student Research Fellowship

Title: Using ultra-short echo time (UTE) MRI to visualize Alzheimer's and cerebrovascular disease pathophysiology

Authors: *J. J. WISCO[1,4], A. NAZARAN[2], D. JEFFS[2], B. HELDT[1], J. KUDLACEK[1], H. W. LAMBERT[5], D. A. MORTON[4], R. K. WATT[3], H. V. VINTERS[6], N. K. BANGERTER[2]; [1]Physiol. and Developmental Biol., [2]Dept. of Electrical and Computer Engineering, Neurosci.

Ctr., [3]Chem. and Biochem., Brigham Young Univ., Provo, UT; [4]Neurobio. and Anat., Univ. of Utah Sch. of Med., Salt Lake City, UT; [5]Neurobio. and Anat., West Virginia Univ. Sch. of Med., Morgantown, WV; [6]Pathology and Lab. Med., David Geffen Sch. of Med. at UCLA, Los Angeles, CA

Abstract: INTRODUCTION: The severity of pathological protein deposition, and concomitant iron presence distinguishes neurological disorders. Tissues with high amounts of protein or iron deposits have a characteristically rapid T2* MRI signal decay. Therefore, these tissue components do not appear on traditional MRI, as the NMR signal has already gone through multiple time constants of decay before any signal can be acquired. Ultra-short Echo Time (UTE) imaging, however, significantly reduces the time between the appearance of an NMR signal and its sampling. The advantage of this rapid signal acquisition is that we can measure the characteristics of the components in the tissues correlated with iron and proteins. In this work, we explored the use of a custom 3D UTE technique to measure very short T2* values within the tissue in ex vivo human brain samples, each with known Braak VI taopathy or with cerebrovascular disease (CVD). We quantified the MR signal from tissues with T2* values of less than 1ms. METHODS: We implemented a 3D UTE MRI sequence with a 3D cones k-space trajectory using a 3T Siemens scanner on a formalin fixed, 20 mm thick, coronal human brain slab from a subject with known Braak VI taopathy, and from a subject with known cerebrovascular disease (CVD). Both slabs included the amygdala and head of the hippocampus, regions with expected deposits of beta amyloid plaques, tau tangles, associated non-heme iron, and possible inflammation. UTE images were acquired at TEs of 0.25, 0.5, 0.8, 1.0, 2.0, 3.0, and 5.0ms and TR of 12.1ms. Resolution was 1 mm isotropic, flip angle was 15 deg, and the FOV was 15 cm in all directions. Difference images were then formed by subtracting the TE=5ms images from the images acquired at the other TEs, effectively suppressing longer T2* tissues. Then, T2* maps of the brains constructed and two ROIs from the same location in hippocampus with short T2* values for both the brains were used for doing histology. RESULTS: We measured T2* values in the amygdala and hippocampus for the ROIs having 63 pixels. T2* values in the ROI of AD brain was 4.8+/-1.9ms (mean+/-SD), and T2* values in the ROI of CVD brain 2.2+/-1.1ms. We analyzed tissue sections for the presence Abeta-42, tau, and CD-68 immunohistochemical reactivity, and enhanced Perl's staining. We noted that the T2* signal decreased with the additive presence of amyloid plaques, tau tangles, non-heme iron, and activated microglia. CONCLUSION: A novel 3D UTE MRI sequence with a 3D cones k-space trajectory was used to image short T2* tissues in the amygdala and hippocampus. Future work will further examine the individual contributions of pathological proteins, non-heme iron, and inflammation to the T2* decay.

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Poster

697. Alzheimer's Disease: Imaging Techniques

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 697.05/T4

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: VA Career Development Award

VA Merit Review

Title: Structural changes to the nucleus basalis of meynert and its projections in the cingulum in mild cognitive impairment

Authors: H. RISKIN-JONES, D. SULTZER, T. NARVAEZ, J. VELIZ, *R. MELROSE;
VA Greater Los Angeles Healthcare Syst., Los Angeles, CA

Abstract: Purpose: The Nucleus Basalis of Meynert (NBM) and its projections in the cingulum are among the first affected structures in Alzheimer's disease (AD). We evaluated the integrity and cognitive correlates of these structures in Mild Cognitive Impairment (MCI), a group at risk for AD. Method: We obtained high-resolution whole-brain T1-weighted images and 64-direction diffusion images from 22 participants with MCI (age: 75.86(8.74), MMSE: 27.23(1.85), 1 female) and 15 elderly controls (EC; age: 74.67 (7.62), MMSE: 29.07 (0.80), 1 female) on a Siemens 3T Skyra. Participants completed a thorough neuropsychological battery. NBM volume was calculated using a previously validated template of basal forebrain subfields. Maps of the cingulum bundle (a major tract in the medial pathway projecting from the NBM) were created on an individual basis using probabilistic tractography in FSL. Average diffusivity measures, including fractional anisotropy (FA), mean diffusivity (MD), and radial diffusivity (RD), were calculated for each subject's cingulum bundles. Results: NBM volume was lower in MCI than EC ($t(35.34)=2.31$, $p<0.05$). Across the entire group, NBM atrophy correlated with poorer verbal memory performance (e.g. logical memory II: $r= 0.41$, $p= 0.01$) and visual memory performance (e.g. BVMT: $r=0.43$, $p < 0.01$), but not hippocampal volume. The diffusivity measures in the cingulum bundle did not differ between groups but were associated with performance on verbal memory tests (e.g. logical memory II with left RD ($r=-0.48$, $p < 0.01$), left MD ($r=-0.33$, $p=0.051$), and left FA ($r=0.46$, $p < 0.01$)), visual memory performance (e.g. BVMT total with left RD ($r= -0.49$, $p < 0.01$), left MD ($r=-0.38$, $p<0.05$) and left FA ($r=0.48$, $p<0.01$)), and hippocampal volume (RD: $r= 0.40$, $p < 0.05$, MD: $r=-0.422$, $p=<0.05$, FA: n.s.). Conclusion: This study used state of the art imaging methods to evaluate the structural integrity of multiple components comprising the cholinergic system: the cell bodies of the NBM, the axons in the cingulum, and targets for cholinergic modulation like the hippocampus. These findings support the cholinergic hypothesis in AD by showing, in vivo, that each component is involved early in the disease process.

Disclosures: **H. Riskin-Jones:** None. **D. Sultzer:** None. **T. Narvaez:** None. **J. Veliz:** None. **R. Melrose:** None.

Poster

697. Alzheimer's Disease: Imaging Techniques

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Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 697.06/T5

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: VA Career Development Award

Title: Executive attention and white matter changes in mild cognitive impairment

Authors: ***A. M. JIMENEZ**, H. RISKIN-JONES, T. NARVAEZ, J. VELIZ, D. L. SULTZER, R. J. MELROSE;
VA Greater Los Angeles Healthcare Syst., Los Angeles, CA

Abstract: Mild cognitive impairment (MCI) is associated with deficits of memory and is generally considered a prodromal stage of Alzheimer's disease (AD). Deficits of attentional control and executive functioning have also been implicated in MCI, and may represent a risk factor for progression to AD. We examined the association between white matter integrity and performance on the color-word Stroop task in 22 patients with MCI (21M, 1F) and 15 elderly controls (14M, 1F). Diagnosis was made by clinician consensus after review of neuropsychological test battery. Each participant completed high resolution MPRAGE and diffusion tensor imaging (DTI, b=1000s/mm2, 64 directions, 2x2x2) scans on a Siemens 3T Skyra. Performance on the Stroop task (Golden version, 45 seconds per condition) was obtained as part of comprehensive neuropsychological testing. Participants with MCI demonstrated intact performance on the Color and Word task conditions, but were impaired relative to controls on the Color-Word condition ($t(35)=2.63$, $p = .01$), and the Color-Word Interference effect ($t(35)=2.59$, $p = .01$). Voxel-wise Tract-Based Spatial Statistics (TBSS) were correlated with the color-word interference effect of the Stroop using Randomise (TFCE applied, uncorrected $p < .02$). We found associations between Stroop performance and white matter integrity (fractional anisotropy; FA) in white matter tracts underlying right dorsolateral prefrontal cortex and superior parietal lobule, as well as right orbito-frontal cortex and insula. These regions correspond to two neural networks associated with the executive control of attention: a dorsal network comprising aspects of lateral frontal and parietal lobes, and a ventral network comprising medial frontal/cingulate regions and anterior insula. These findings suggest difficulties in MCI with both top-down and bottom-up attentional processing, impacting task-switching and conflict-monitoring abilities. They also join a growing body of work showing that AD-associated

pathology is not limited to the cortex, but includes alterations to white matter. Results point to potential mechanism for pathophysiology of early cognitive deficits leading to AD, and a potential imaging biomarker of early AD-related brain changes.

Disclosures: A.M. Jimenez: None. **H. Riskin-Jones:** None. **T. Narvaez:** None. **J. Veliz:** None. **D.L. Sultzer:** None. **R.J. Melrose:** None.

Poster

697. Alzheimer's Disease: Imaging Techniques

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 697.07/T6

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Permeability in healthy people

Authors: B. PASCUAL[1], E. ROCKERS[1], S. BAJAJ[1], M. YU[1], C. KARMONIK[1], Z. XUE[1], *J. C. MASDEU[2,1];
[1]Houston Methodist Res. Institute, Weill Cornell Med. Col., Houston, TX; [2]Section on Integrative Neuroimaging, NIH, Washington, DC

Abstract: Vascular Permeability in Substantia Nigra, Globus Pallidus, Putamen and Thalamus is Higher in Older as Compared to Younger Subjects
Background: In older healthy subjects, [^{18}F]AV-1451, a PET tau tracer that is supposed to bind to hyperphosphorylated tau, seems to bind to regions (putamen and other nuclei) known not to have abnormal tau deposition on neuropathology. Our aim was to determine whether apparently increased specific [^{18}F]AV-1451 binding in areas unlikely to harbor hyperphosphorylated tau, such as the substantia nigra, thalamus, globus pallidus and putamen, of older subjects could be related to greater vascular permeability of these regions in older subjects as compared to younger ones.
Methods. In six younger (23±2.1 years of age, three women) and six older (68.8±7.6 years, three women) healthy subjects we measured dynamic [^{18}F]AV-1451 uptake over a three-hour period. In the same subjects we obtained dynamic gadolinium concentrations before and after a bolus injection of gadolinium, a large molecule commonly used to assess the permeability of the blood-brain barrier, by performing a pre-contrast 3D T1 map (five flip angle acquisitions) followed by a dynamic contrast enhanced MRI (DCE-MRI). During and after infusion, we acquired 180 consecutive T1-weighted volumes (3.4 sec per volume) over 10 minutes. SUVr of [^{18}F]AV-1451 and permeability parameters (K*trans*) of gadolinium were analyzed by volume-of-interest methods.
Results. There was greater SUVR of [^{18}F]AV-1451 in the thalamus, globus pallidus and

putamen (but not in cerebellum or cerebral cortex) of older as compared to younger subjects. Gadolinium K*trans* was similar in the cerebellum, temporal cortex, and choroid plexus of younger and older subjects, but there was higher permeability in the substantia nigra, thalamus, globus pallidus, and putamen (all $p<0.05$) of older as compared to younger subjects.
Conclusion. Increased capillary permeability in substantia nigra, pallidum and putamen of healthy older subjects could underlie some of the regional differences in [^{18}F]AV-1451 uptake observed in older as compared to younger healthy subjects.

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Poster

697. Alzheimer's Disease: Imaging Techniques

Location: Halls B-H

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Program#/Poster#: 697.08/T7

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Leading-edge indicators of network disease spread in frontotemporal dementia.

Authors: *J. BROWN, J. DENG, M. GORNO-TEMPINI, H. J. ROSEN, W. W. SEELEY; Univ. of California San Francisco, San Francisco, CA

Abstract: In frontotemporal dementia syndromes including behavioral variant frontotemporal dementia (bvFTD) and semantic variant primary progressive aphasia (svPPA), neurodegeneration appears to originate in a selectively vulnerable epicenter region and spread outward via network connections. This model has been tested in cross sectional data but has not been validated with longitudinal data. In this study we developed a new unified model for predicting both cumulative regional atrophy and regional atrophy rate of change as a function of path length from the epicenter and disease stage.
We modeled cumulative gray matter atrophy over time in each region as its own sigmoidal function, where the sigmoid onset time is determined by network path length from the epicenter. If cumulative atrophy increases sigmoidally, the rate of change will follow the sigmoidal derivative curve. The main prediction of this model is that regional longitudinal atrophy rate will peak before cumulative atrophy plateaus, and thus rate of change will reveal the “leading edge” of disease spread earlier than cumulative atrophy.
We tested this model in patients with bvFTD (n=17), svPPA (n=22), and age-matched controls (n=26) T1 MRI scanned twice 12 months apart. A whole brain parcellation of 482 regions was

used to extract svPPA/bvFTD regional cumulative atrophy scores and longitudinal atrophy scores. We identified the syndrome epicenters in the anterior temporal lobe/frontoinsula from our previous study. Next, a healthy functional 482x482 connectome was used to determine each region's shortest path length from the epicenter. We built a model with sigmoid/derivative curves for each region based on path length, and then predicted the cumulative/longitudinal atrophy for each region by sampling each region's sigmoid/derivative curves at a fixed timepoint. We then tested the accuracy of this model against our observed data.
The combined model of spread in bvFTD from the epicenter in the right frontoinsula was significant for both cumulative atrophy ($r = 0.42$, $p < 0.0001$) and longitudinal rate of atrophy ($r = 0.30$, $p < 0.0001$). In svPPA, the model of spread from the left temporal pole was significant for cumulative atrophy ($r = 0.70$, $p < 0.0001$) and longitudinal rate of atrophy ($r = 0.38$, $p < 0.0001$). Thus, a network-based model making joint predictions about cumulative atrophy and rate of change can unify observations from baseline and longitudinal structural MRI studies, while providing a more accurate prognosis about downstream regions at greatest risk during disease progression.

Disclosures: J. Brown: None. **J. Deng:** None. **M. Gorno-Tempini:** None. **H.J. Rosen:** None. **W.W. Seeley:** None.

Poster

697. Alzheimer's Disease: Imaging Techniques

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant TL1 TR001107

NIH Grant UL1 TR001108

Title: Resting state network profiles of alzheimer's disease and frontotemporal dementia: preliminary examination

Authors: *J. A. CONTRERAS[1], S. L. RISACHER[2], M. DZEMIDZIC[2], J. D. WEST[2], B. C. MCDONALD[2], M. R. FARLOW[3], B. MATHEWS[3], L. G. APOSTOLOVA[3], J. BROSCH[4], B. GHETTI[5], J. GOÑI[6], O. SPORNS[7], A. J. SAYKIN[2];
[2]Radiology and Imaging Sci., [3]Dept. of Neurol., [1]Indiana Univ. Sch. Of Medicine, STARK, Indianapolis, IN; [4]Dept. of Neurol., [5]Indiana Univ. Sch. Of Med., Indianapolis, IN; [6]Col. of Industrial Engin. and Biomed. Engin., Purdue Univ., West Lafayette, IN; [7]Dept. of Psychological and Brain Sci., Indiana Univ., Bloomington, IN

Abstract: The assessment of resting-state functional connectivity (FC) has become an important tool in studying brain disorders. Here we define functional connectivity as correlations of brain activity between two or more anatomically distinct brain regions with data obtained from resting-state functional magnetic resonance imaging (rsfMRI). These brain regions can be classified as components of well-established resting state networks (RSNs). Recent evidence from rsfMRI studies have shown that brain network connectivity is altered in patients with neurodegenerative disorders. However, few studies have examined the complete connectivity patterns of these well-reported RSNs using a whole brain approach and how they compare between dementias. Here, we used advanced connectomic approaches to examine the connectivity of RSNs in Alzheimer's disease (AD), Frontotemporal dementia (FTD), and age-matched control participants.22 participants (10 controls [63.4±7.8 years], 8 AD [72.63±13.9 years], 4 FTD [62.75±8.1 years]) from an ongoing study at Indiana University School of Medicine underwent a ten minute rsfMRI session (eyes closed) on a Siemens MRI ^{3}T Prisma Scanner. Data was processed using an in-house pipeline modeled after Power et. al., 2014. Images were parcellated into 278 regions of interest (ROI) based on Shen et. al., 2013. Connectivity between each ROI pair was described by Pearson's correlation coefficient. Brain regions were grouped into seven canonical RSNs as described by Yeo et al. (2015). Pearson correlation values were then averaged across pairs of ROIs in each network and averaged across individuals in each group. These values were used to determine relative expression of FC in each RSN (intra-network) and create RSN profiles for each group. AD and FTD exhibited a similar profile of RSNs, showing higher FC within somatomotor, ventral attention, limbic, frontoparietal, and DMN networks compared to controls. However, intra-network FC in AD was lower for all networks compared to FTD. While previous studies noted changes in network patterns in specific neurodegenerative states, few have examined the relative expression of FC in each RSN using a whole brain connectivity approach. Therefore, our approach allows us to create profiles that could help compare intra-network FC in different neurodegenerative diseases. Future work with expanded samples will help us to draw more substantial conclusions regarding differences, if any, in the connectivity patterns between RSNs in various neurodegenerative diseases.

Disclosures: **J.A. Contreras:** None. **S.L. Risacher:** None. **M. Dzemidzic:** None. **J.D. West:** None. **B.C. McDonald:** None. **M.R. Farlow:** None. **B. Mathews:** None. **L.G. Apostolova:** None. **J. Brosch:** None. **B. Ghetti:** None. **J. Goñi:** None. **O. Sporns:** None. **A.J. Saykin:** None.

Poster

697. Alzheimer's Disease: Imaging Techniques

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Program#/Poster#: 697.10/T9

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: LECMA Grant 14749

Fondation Jérôme Lejeune

Title: Early endosome aggregation in Down Syndrome revealed by high-resolution microscopy

Authors: *A. BOTTÉ[1], A. FRANCK[1,2], J. LAINÉ[1], G. FONTAINE[1], F. CORLIER[1,3], C. ALBAC[1], P. GOH[4], O. FAKLARIS[5], A.-S. REBILLAT[6], D. NIZETIC[4], M.-C. POTIER[1];
[1]ICM - Inst. Du Cerveau Et De La Moelle Épinière, Paris, France; [2]Inst. de Myologie, Paris, France; [3]Inst. for Neuroimaging and Informatics (INI), USC, Los Angeles, CA; [4]Blizard Inst., Queen Mary, Univ. of London, London, United Kingdom; [5]Imagoseine, Inst. Jacques Monod, Paris, France; [6]Inst. Jérôme Lejeune, Paris, France

Abstract: Early morphological alterations of subcellular organelles from the endo-lysosomal pathway in Alzheimer's disease (AD) and Down Syndrome (DS) were widely observed by immunocytochemistry and confocal microscopy, notably an increase in size of early endosomes (EE). Neurons bearing enlarged EE have been described before amyloid pathology and clinical symptoms (Cataldo et al., 2000). Endosomal enlargement was also detected in primary fibroblasts of individuals with DS (Jiang et al., 2010, Cataldo et al., 2008), peripheral blood mononuclear cells from individuals with DS and AD patients (Cossec et al., 2012; Corlier et al., 2015), in the brain of mouse models of familial AD with mutations in the gene encoding APP (Choi et al., 2013) and in the brain of Ts65Dn mice modeling DS (Cataldo et al., 2003).
EE diameter ranges from 100 to 250 nanometers, making their observation hampered by the diffraction-limited resolution of conventional light microscopy which is the only technique used so far to investigate EE morphological alterations. In the present work we used super-resolution microscopy and ultrastructural imaging to further characterize the endosomal compartment in cellular and mouse models of DS.
We immunostained EE using an anti-EEA1 antibody (C45B10, Cell Signaling) in cells from individuals with DS (lymphoblastoid cell lines and fibroblasts) and in basal forebrain cholinergic neurons (BFCNs) from Ts65Dn brains. Electron microscopy revealed clusters of normal-sized EE in lymphoblastoid cell lines (EE mean area: control = 0.0538μm^2 vs. DS = 0.0473μm^2, t-test p-value = 0.52), in fibroblasts and in BFCNs from Ts65Dn (1.98-fold increase of endosomal clusters as compared to control mice), suggesting that previously described enlarged EE are rather aggregated EE. We next analyzed EE in neurons derived from induced pluripotent stem cell clones of an individual with a mosaic trisomy 21 (T21). We observed enlarged EE by widefield microscopy in neurons with T21 as compared to euploid neurons (mean volume: 0.017μm^3 vs. 0.021μm^3 respectively, t-test p-value = 0.01). Super-resolution structured illumination microscopy observations revealed that these apparently enlarged EE corresponded to clusters of normal-sized EE (mean volume: control = 0.00801μm^3 vs. DS = 0.0079μm^3, t-test p-value = 0.84).
Our work confirms the significance of EE dysfunction in DS as previously demonstrated in numerous studies. Furthermore, using high resolution microscopies we unveil the presence of aggregates of normal-sized EE rather than enlarged EE. This new result implies to redirect the effort made on the understanding of EE abnormalities and their implication in DS and AD pathogenesis.

Disclosures: **A. Botté:** None. **A. Franck:** None. **J. Lainé:** None. **G. Fontaine:** None. **F. Corlier:** None. **C. Albac:** None. **P. Goh:** None. **O. Faklaris:** None. **A. Rebillat:** None. **D. Nizetic:** None. **M. Potier:** None.

Poster

697. Alzheimer's Disease: Imaging Techniques

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 697.11/T10

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Live cell visualization of G2 phase transition in neurons driven by amyloid-β using a fluorescent ubiquitination-based cell cycle indicator (Fucci) system

Authors: *S. IPPATI;
UNSW, Unsw Sydney, Australia

Abstract: Cell cycle proteins are reported to be expressed in neurons in Alzheimer's disease (AD) (Yang et al., 2003). Despite supporting evidence that amyloid-β (Aβ) and tau-mediated toxicity is accompanied by neuron progression in the G_1, S or G_2 phase of the cell cycle, ectopic mitosis events have yet to be shown (Copani et al., 1999; Giovanni et al., 1999). Therefore, cell cycle re-entry events are hypothesized to contribute to neurodegeneration in mature neurons and the failure to complete the cell cycle results in neuronal cell death (Raina et al., 2001). The potential role of cell cycle proteins in neurodegenerative diseases associated with neuronal loss is still mechanistically unknown. Likewise, the temporal role of (Aβ) in inducing aberrant cell cycle reentry is also unknown. Here, we used a method designed to track cell-cycle progression described from Sakaue- Sawano and colleagues (2008). This fluorescent ubiquitination-based cell cycle indicator (Fucci) system allows the tracking of the G_1-G_2/S phase transition in living cells by exploiting the cell-cycle dependent proteolysis of the ubiquitination oscillators Cdt1 and Geminin. The fusion of the red- and green-emitting fluorescent proteins mKO2 and Azami Green (mAG) to portions of Cdt1 and Geminin respectively in the nuclei of cells causes cells in G_1 to emit red fluorescence and those in S/G_2/M phase to emit green fluorescence. We optimized this method in primary neurons to allow simultaneous tracking of the progression of live cells in the G_2 phase and the occurrence of cell death after the administration of different preparations of Aβ. It was shown that primary hippocampal neurons transduced via lentivirus with both the mKO2 and mAG fucci constructs re-enter the cell cycle in response to Aβ peptides administration. We also show that the number of neurons entering the G_2 phase of the cell cycle increases with longer incubation times and higher concentrations of Aβ. We did not observe any neurons in the G_2 phase in the untreated controls, suggesting that the G_1 to G_2 transition is an Aβ

dependent event. There were also no changes in the cell morphology or rate of cell death in response to Aβ treatment. We have used this system to dynamically study the neuronal cell behavior and to quantify the numbers of neurons that re-enter the cell cycle in response to oligomeric, fibrillary and monomeric forms of β-amyloid peptides. The physiological relevance of this *in vitro* cell based model is underscored by this data accurately reflecting those previously observed *in vivo* where we observed that Aβ expression in mice is associated with the expression of several cell cycle proteins in the brains of APP transgenic mice.

Disclosures: S. Ippati: None.

Poster

698. Modeling Parkinson's Disease

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 698.01/T11

Topic: C.03. Parkinson's Disease

Title: Acceleration of synuclein pathology induced by seeding with recombinant preformed α-synuclein fibrils

Authors: *D. L. CZILLI, L. LI, M. HAYASHI, Y. TIAN;
Lilly Res. Labs, Eli Lilly and Co., Indianapolis, IN

Abstract: Parkinson's disease (PD) is pathologically characterized by a progressive loss of dopamine neurons in the substantia nigra, and aggregation of the α-synuclein (α-Syn) protein into Lewy bodies (LB). A53T (B6;C3-Tg(Prnp-SNCA*A53T)83Vle/J) is a transgenic animal model that expresses human A53T variant alpha-synuclein (full-length, 140 amino acid isoform) under the direction of the mouse prion protein promoter. Our experience with this model has shown that it can take 8 to 10 months for a subset of homozygous mice to develop the severe motor phenotype and concomitantly robust pathology primarily centered in the brainstem and spinal cord. Once pathology arises, those animals become moribund and die within a matter of days. To establish a more consistent symptomatic model, we performed intracerebral inoculation of pathological α-synuclein using recombinant preformed α-synuclein fibril seeds (sonicated PFFs) in 3 and 7 month old male Tg A53T mice. This inoculation initiated a rapid progression and spreading of α-synucleinopathy as described by Luk et al., 2012. At 90 days post infusion (dpi), we saw widespread LB-like α-synuclein pathology not only limited to the injection site, but also abundant on the contralateral side and other interconnected regions of brain stem, cortex and spinal cord in both 3 and 7 month old mice. The age of mice at the time of inoculation did not make a significant difference in pathology development. In addition, we developed biochemical methods to quantitatively examine the a-synuclein propagation. Phosphorylated and

mis-folded α-synuclein in spinal cord lysates were measured by ELISA and western blot and we showed a significant increase of both phosphorylated and mis-folded α-synuclein in the PFF-injected spinal cord lysates compared to a-synuclein monomer injected controls. Using this model, we were able to demonstrate that inoculation with synthetic α-Syn preformed fibrils (PFFs) can accelerate the formation and propagation of pathological inclusions throughout the mouse CNS. In addition, the inclusions formed appear consistent with those seen in PD-like α-Syn pathology.

Disclosures: **D.L. Czilli:** A. Employment/Salary (full or part-time): Eli Lilly and Co. **L. Li:** A. Employment/Salary (full or part-time): Eli Lilly and Co. **M. Hayashi:** A. Employment/Salary (full or part-time): Eli Lilly and Co. **Y. Tian:** A. Employment/Salary (full or part-time): Eli Lilly and Co..

Poster

698. Modeling Parkinson's Disease

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 698.02/T12

Topic: C.03. Parkinson's Disease

Support: Intramural research program of the national institute on aging

Title: Chronic gut inflammation hastens the onset of motor dysfunction and pathology in alpha-synuclein mutant transgenic mice.

Authors: *Y. KISHIMOTO[1,2], W. ZHU[3], J. M. SEN[3], M. P. MATTSON[2];
[2]Lab. of Neurosciences, Natl. Inst. on Aging Intramural Res. Program, [3]Immune Cells and Inflammation Section, Lab. of Immunology, Natl. Inst. on Aging,, [1]NIH, Baltimore, MD

Abstract: Emerging evidence from studies of human subjects and animal models suggest that Parkinson's disease (PD) pathology (alpha-syunclein accumulation) and neuronal dysfunction may occur first in peripheral neurons of the autonomic nervous system including the enteric branches of the vagus nerve (for review see Del Tredici and Braak; *Neuropathol Appl Neurobiol.* 2016; 42:33-50). The risk of PD increases greatly in people over the age of 65, a period of life in which chronic inflammation is common in many organ systems including the gut. We previously reported that a pro-inflammatory diet (high fat, glucose and fructose) exacerbated autonomic (vagal parasympathetic) dysfunction, whereas an anti-inflammatory diet (alternate day fasting) ameliorated vagal dysfunction in transgenic mice overexpressing mutant human alpha-synuclein (A53T PD mice) (Griffioen et al., *Neurobiol Aging*. 2013; 34:928-935). In the present study we are testing the hypothesis that chronic low-level intestinal inflammation can accelerate the age of

disease onset, and worsen alpha-synuclein pathology and systemic and CNS inflammation in A53T alpha-synuclein mutant transgenic mice. Wild type (WT) and A53T PD mice were treated with or without 0.5% (w/v) dextran sodium sulphate (DSS) in their drinking water for 12 weeks beginning at 3 months of age. During the first 2 weeks of DSS treatment both WT and A53T PD modest weight loss and intermittent diarrhea which subsequently subsided. At 12 weeks of treatment the WT/DSS and A53T PD/DSS groups exhibited colon shortening and spleen enlargement compared with control mice that did not receive DSS. The age of onset of motor dysfunction, evaluated using a rotarod test, gait analysis and grip strength measurements, was significantly earlier in DSS-treated A53T PD mice compared to control A53T PD mice. Markers of systemic inflammation were also increased in DSS-treated A53T PD compared to A53T PD mice in the control group. We are currently evaluating alpha-synuclein pathology in the gut, vagus nerve and brain. These findings from a mouse model suggest the possibility that chronic gut inflammation can promote pathogenic processes in PD, and are consistent with the notion that the disease can be initiated in peripheral autonomic neurons and then progress into the brain a retrograde manner. Supported by the Intramural Research Program of the National Institute on Aging.

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Poster

698. Modeling Parkinson's Disease

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Program#/Poster#: 698.03/T13

Topic: C.03. Parkinson’s Disease

Support: Brain Mapping by Integrated Neurotechnologies for Disease Studies (Brain/MINDS)

Strategic Research Program for Brain Science from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (MEXT) and Japan Agency for Medical Research and Development (A-MED)

MEXT Grant FY2014-2018

Title: Mutated α -Synuclein transgenic marmosets as a novel non-human primate model of Parkinson’s disease

Authors: ***R. KOBAYASHI**[1], S. SHIOZAWA[1], J. OKAHARA[2], C. YOKOYAMA[3], T. KONDO[1], J. TAKAHASHI-FUJIGASAKI[4], T. INOUE[2], C. HARA-MIYAUCHI[5], T. MAEDA[1], H. J. OKANO[6], E. SASAKI[2], H. OKANO[1];

[1]Keio Univ. Sch. of Med., Tokyo, Japan; [2]Central Inst. for Exptl. Animals, Kanagawa, Japan; [3]Div. of Bio-function Dynamics Imaging, RIKEN Ctr. for Life Sci. Technologies, Hyogo, Japan; [4]Dep. of Neuropathology, Brain Bank for Aging Res., Tokyo Metropolitan Inst. of Gerontology, Tokyo, Japan; [5]Div. of Regenerative Med., [6]Jikei Univ. Sch. of Med., Tokyo, Japan

Abstract: Parkinson's disease (PD) is a neurodegenerative disease characterized by loss of dopaminergic neurons in the substantia nigra that causes tremor, akinesia and muscle rigidity. A pathological hallmark of PD brain is the appearance of Lewy bodies, which is comprised of more than 70 molecules. Among them, α-Synuclein is regarded as a major component of the Lewy body and one cause of the PD. In fact, mutations in SNCA gene encoding α-Synuclein cause familial PD. Nevertheless, the precise function of the α-Synuclein in PD onset is unclear. Although transgenic mouse models of PD carrying mutated α-Synuclein have been established so far, these models could not reproduce proper PD pathology because of differences in brain structure and function. This has been one of the major limitations for PD research. Hence, MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine)-induced PD models in non-human primates, which show phenotypes similar to progressive stage PD patient, have been widely used. However, it is still difficult to investigate phenotypes before onset or at early stages using this drug-induced non-human primate model. From this point of view, transgenic models of non-human primate are desired. In this presentation, we report establishment and analysis of transgenic common marmosets, a small non-human primate, harboring mutated α-Synuclein.

Disclosures: R. Kobayashi: None. **S. Shiozawa:** None. **J. Okahara:** None. **C. Yokoyama:** None. **T. Kondo:** None. **J. Takahashi-Fujigasaki:** None. **T. Inoue:** None. **C. Hara-Miyauchi:** None. **T. Maeda:** None. **H.J. Okano:** None. **E. Sasaki:** None. **H. Okano:** None.

Poster

698. Modeling Parkinson's Disease

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Program#/Poster#: 698.04/T14

Topic: C.03. Parkinson’s Disease

Support: NIH Grant 1P50NS071669

Title: A novel transgenic mouse model to investigate Parkinson's disease-like alpha-synuclein pathology in noradrenergic neurons

Authors: *L. M. BUTKOVICH, M. C. HOUSER, K. KHOSRAVIAN, T. CHALERMPALANUPAP, J. CHANG, D. WEINSHENKER, M. G. TANSEY;
Emory Univ., Atlanta, GA

Abstract: While cell loss and α-synuclein (αsyn) aggregates in the substantia nigra pars compacta (SNpc) are a major hallmark of Parkinson's disease PD, pathology in the locus coeruleus (LC) is commonly more severe, and may even precede that found in the SNpc and contribute to nigrostriatal loss. While most PD research has focused on the SNpc, we have lacked suitable models to understand how αsyn pathology specifically affects noradrenergic neurons in PD, and whether noradrenergic neurons are vulnerable to αsyn pathology. Increased expression of αsyn is a factor in its aggregation, as heritable triplication mutations in the *SNCA* gene are associated with early-onset PD. Transgenic models of αsyn overexpression have previously been unable to selectively target LC neurons, limiting our understanding of how αsyn pathology affects noradrenergic neurons. To examine this question, we have developed a BAC-transgenic mouse model overexpressing wild-type human αsyn under the control of the noradrenergic-specific dopamine β-hydroxylase promoter. These animals overexpress human αsyn in LC neurons, and enteric neurons derived from the neural crest. Preliminary analysis revealed human αsyn immunoreactivity and mRNA expression in noradrenergic neurons of the LC in transgenic mice, but not non-transgenic littermates. We are currently evaluating age-dependent LC neuron loss and fiber degeneration in these mice. In the transgenic gastrointestinal system there is a significant increase in αsyn expression in enteric neurons at 14 months, raising the possibility that the model may also prove useful for examining peripheral pathologies commonly seen in PD in the gastrointestinal tract related to αsyn accumulation. Combined, our preliminary findings indicate that this novel transgenic mouse model overexpresses human αsyn in noradrenergic neurons, and that it could provide insight into the mechanisms underlying PD-like αsyn aggregation and spread, as well as a platform to test future therapeutic strategies to target αsyn pathology in regions other than the SNpc.

Disclosures: L.M. Butkovich: None. **M.C. Houser:** None. **K. Khosravian:** None. **T. Chalermpalanupap:** None. **J. Chang:** None. **D. Weinshenker:** None. **M.G. Tansey:** None.

Poster

698. Modeling Parkinson's Disease

Location: Halls B-H

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Program#/Poster#: 698.05/T15

Topic: C.03. Parkinson's Disease

Support: Supported by PAPIIT-DGAPA-UNAM IN215114

Title: Cytological andbehavioral evaluation in male mice before, during and after Manganese chlorideand Manganese acetate inhalation as a Parkinson disease experimental model

Authors: F. HUERTA-OLIVAREZ[1], E. MONTIEL-FLORES[1], *L. REYNOSO-ERAZO[2], V. ANAYA-MARTÍNEZ[1], J. SANCHEZ-BETANCOURT[1], J. ESPINOSA-VILLANUEVA[1], C. LOZANO-VILLALOBOS[1], M. AVILA-COSTA[1];
[1]Neurosci., UNAM, Neuromorphology Lab., Mexico, Mexico; [2]Univ. of Mexico, Tlalnepantla Edo Mex, Mexico

Abstract: Manganese (Mn) is an essential metal that is found in all tissues, it has an important function in different metabolism processes and serves as a cofactor for many enzymes. It is known that Mn is essential for development and brain function; however, at high concentrations this metal is neurotoxic and concentrates mainly in the Basal Ganglia (BG), where developed a neurological disorder similar to Parkinson Disease (PD), referred to as Manganism. The clinical symptoms of Mn neurotoxicity include: psychiatric disorders, parkinsonism and dystonia, if the exposure continues and the disease progresses, the patients develop hypokinesia, rigidity and muscle tremor. In our laboratory was characterized a PD model through the inhalation of Mn^{+2} and Mn^{+3} that produces ultrastuctural changes, dopaminergic neurons degeneration in the Substancia Nigra pars compacta, (SNc) and motor impairments, the alterations are progressive and bilateral, which makes it advantageous to other models, so this study examines the motor and cytological changes in male mice while inhaled the Mn mixture (manganese chloride ($MnCl_2$) and Mn acetate ($Mn(OAc)_3$) for 7 months and compare with mice which inhaled for 5 months and were left 2 more months to determinate if the motor impairments improve after Mn inhalation has concluded. The experiments were carried out in CD1 male mice, divided into 3 groups: 10 mice (control) were exposed to deionized water, 10 mice were exposed to the Mn mixture for 7 months (continue inhalation) and 10 mice were exposed to Mn for 5 months and were left without inhalation for two months (post inhalation). The inhalation was performed in an acrylic box 1 hour 2 times per week. Every week, the motor performance was evaluated, in the walking beam and in the reaching task tests. After 7 months, the animals were sacrificed, perfused and the brains were extracted, we performed immunohistochemistry for tyrosine hydroxylase (TH), to count SNc TH-immunoreactive neurons. According to our results the exposition to the Mn mixture induced motor alterations, in both, Reaching Task and beam walking test, in addition to the loss of dopaminergic neurons of approximately 67 % in exposed animals. There were no recovery in the exposed groups for 5 months, in comparison to the continued exposition group (7 months) so we considerer that the inhalation of Mn compounds is a reliable model that simulates some behavioral and morphological disturbances as reported in PD since it is bilateral, progressive, non invasive, and the animals did not recover after the inhalation has been terminated and is easily reproduced.

Disclosures: F. Huerta-Olivarez: None. **E. Montiel-Flores:** None. **L. Reynoso-Erazo:** None. **V. Anaya-Martínez:** None. **J. Sanchez-Betancourt:** None. **J. Espinosa-Villanueva:** None. **C. Lozano-Villalobos:** None. **M. Avila-Costa:** None.

Poster

698. Modeling Parkinson's Disease

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 698.06/T16

Topic: C.03. Parkinson's Disease

Support: Medical Support Fund of Parkinsonism with Dementia, Japan Foundation for Neuroscience and Mental Health

Title: Generation of Parkinson's disease mice model by temporarily restricted expression of α-synuclein using Tet-off system

Authors: *H. YAMAGUCHI[1], A. FUJITA[1], K. IINUMA[1], H. UNE[1], Y. ZHAO[1], K. TANAKA[2], J.-I. KIRA[1];
[1]Dept. of Neurol., Neurolog. Inst, Kyushu Univ. Sch. of Med., Fukuoka-Shi, Japan; [2]Dept. of Neuropsychiatry, Keio Univ. Sch. of Med., Tokyo, Japan

Abstract: To investigate the molecular mechanism of dopaminergic (DA) neuron death related to α-synuclein pathology and to establish Parkinson's disease mice model, we aimed to establish mice, in which α-synuclein is overexpressed in DA neurons in a temporarily restrictive manner using Tet-off system. We used TetO-α-SynA53TTg/+ mice and DAT-tTA Tg/+ mice. TetO-α-SynA53TTg/+ mice express mutant A53T human α-synuclein (α-SynA53T) regulated by a tetracycline operator. DAT-tTA Tg/+ mice express a tetracycline-controlled transactivator protein (tTA) driven by the mouse dopamine transporter (DAT) promoter. We mated TetO-α-SynA53TTg/+ mice with DAT-tTA Tg/+ mice and analyzed their offspring including TetO-α-SynA53TTg/+;DAT-tTA Tg/+, TetO-α-SynA53TTg/+;DAT-tTA +/+, TetO-α-SynA53T+/+;DAT-tTA Tg/+, and TetO-α-SynA53T +/+;DAT-tTA +/+ mice. Immunohistochemical analysis showed that TetO-α-SynA53T Tg/+;DAT-tTA Tg/+mice successfully expressed α-SynA53T in DA neurons in the SN in the absence of doxycycline (Tet-off). These mice exhibited DA neurons loss in the SN in adulthood. TetO-α-SynA53T Tg/+;DAT-tTA Tg/+ mice are useful for the investigation of the molecular mechanism of DA neurons death in Parkinson's disease.

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Poster

698. Modeling Parkinson's Disease

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 698.07/T17

Topic: C.03. Parkinson's Disease

Title: AAV-mediated expression of alpha-synuclein in oligodendrocytes recapitulates key features of multiple system atrophy

Authors: F. BASSIL[1], P. A. GUERIN[1], N. DUTHEIL[1], S. DOVERO[1], W. G. MEISSNER[1,2,3], E. BEZARD[1], *P.-O. FERNAGUT[1];
[1]Inst. Des Maladies Neurodegeneratives, Bordeaux, France; [2]Service de Neurologie, [3]Ctr. de référence atrophie multisystématisée, CHU de Bordeaux, Bordeaux, France

Abstract: Multiple system atrophy (MSA) is a fatal neurodegenerative disorder characterized by a combination of autonomic dysfunction, cerebellar ataxia and l-dopa unresponsive parkinsonism. The hallmark of MSA is the accumulation of α-synuclein (α-syn) aggregates in oligodendrocytes forming glial cytoplasmic inclusions. Currently available models of MSA are transgenic mice with constitutive expression of α-syn in oligodendrocytes. These models replicate several features of MSA but do not display progressive and robust neurodegeneration in brain regions severely affected in MSA such as the striatum and substantia nigra. Adeno-associated viruses (AAV) allow targeting the expression of disease-associated genes in selected cellular ensembles and have proven useful to improve the modelling of Parkinson's disease by targeting the neuronal overexpression of α-syn in the substantia nigra of rodents and non-human primates. Here, we developed chimeric AAV1/2 vectors expressing either human wild-type α-syn or the green fluorescent protein (GFP) under the control of the mouse myelin basic protein (MBP) promoter. MBP-driven expression of GFP resulted in >80% oligodendroglial selectivity in rats and mice. Bilateral injection of AAV expressing α-syn in the striatum of rats resulted in progressive bilateral stepping deficits that were not improved by l-dopa when assessed 6 months after AAV-injection. Histopathological analysis revealed a significant loss of dopaminergic neurons at 3 months, further progressing at 6 months, together with a reduction of striatal medium spiny neurons. Prominent α-syn accumulation, including pS129 and proteinase-K resistant α-syn was detected in the striatum and substantia nigra. Histopathological assessment of AAV-injected monkeys is currently underway. AAV-mediated oligodendroglial expression of α-syn in rats allows inducing progressive and l-dopa unresponsive sensorimotor impairments associated with progressive nigral and striatal neurodegeneration, and accumulation of pathologically relevant forms of α-syn, thereby replicating some of the key features of MSA. This flexible strategy can be used to investigate, in several species, how α-syn accumulation in selected oligodendroglial populations contributes to the pathophysiology of MSA. This AAV-

mediated modelling of MSA expands the currently limited range of α-syn-based models and offers a new framework for the pre-clinical validation of therapeutic strategies.

Disclosures: **F. Bassil:** None. **P.A. Guerin:** None. **N. Dutheil:** None. **S. Dovero:** None. **W.G. Meissner:** None. **E. Bezard:** None. **P. Fernagut:** None.

Poster

698. Modeling Parkinson's Disease

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Topic: C.03. Parkinson's Disease

Support: Supported by PAPIIT-DGAPA-UNAM IN215114

Title: Progression & lateralization of the dopaminergic degeneration in mice exposed to manganese

Authors: C. LOZANO-VILLALOBOS[1], V. ANAYA-MARTINEZ[1], E. MONTIEL-FLORES[1], E. MONTIEL-FLORES[1], J. SANCHEZ-BETANCOURT[1], F. HUERTA-OLIVAREZ[1], J. ESPINOSA VILLANUEVA[1], L. REYNOSO-ERAZO[1], *M. AVILA-COSTA[2];
[1]Neurosci., UNAM, Neuromorphology Lab., Mexico, Mexico; [2]UNAM, Neuromorphology Lab., Tlalnepantla Edo Mex, Mexico

Abstract: Parkinson's disease (PD) is a neurodegenerative disorder characterized by motor symptoms such as resting tremor, bradykinesia, rigidity and muscle weakness. These changes result from the loss of dopaminergic neurons of the substantia nigra compacta (SNc). It is known that chronic exposure to high levels of manganese (Mn) tends to accumulate in the brain, especially in the basal ganglia, mainly in dopamine (DA) rich regions, altering the integrity of dopaminergic neurons and DA neurochemistry, resulting in a neurological syndrome resembling PD. The present study was designed to determine neuronal death laterality and progression of SNc dopaminergic neurons in mice exposed to inhaled divalent and trivalent manganese (Mn2+/Mn3+) mixture at different times. CD-1 male mice inhaled a mixture of 0.04 M manganese chloride (MnCl2) and 0.02 M manganese acetate (Mn(OAc)3), 1 h twice a week for 15 days, a month and also for two, three, four and five months. By the end of Mn exposure period, animals were killed. The mesencephalon was processed for tyrosine hydroxylase (TH) immunocytochemistry. After two months of Mn mixture inhalation the number of TH-immunopositive neurons in SNc decreased 25% and 42%, 51% and 61% at three, four and five months, respectively. We also note that the number of dopaminergic neurons was greater on the left side. This difference was significant only at two and three months after starting the inhalation

showing a loss of 16% and 14% for three months. These data provide evidence that Mn mixture inhalation produces alterations similar to those seen in PD since the death of dopaminergic neurons is bilateral, lateralized, gradual and progressive.

Disclosures: C. Lozano-Villalobos: None. **V. Anaya-Martinez:** None. **E. Montiel-Flores:** None. **E. Montiel-Flores:** None. **J. Sanchez-Betancourt:** None. **F. Huerta-Olivarez:** None. **J. Espinosa Villanueva:** None. **L. Reynoso-Erazo:** None. **M. Avila-Costa:** None.

Poster

698. Modeling Parkinson's Disease

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Program#/Poster#: 698.09/U1

Topic: C.03. Parkinson's Disease

Support: NIH Grant P20GM103408

NIH Grant P20GM109095

The Biomolecular Research Center at Boise State

Title: Discovery of nigral dopaminergic neurogenesis in adult mice

Authors: *B. MORRISON, A. RAHMAN, J. ALBRIGHT, I. STOJKOVSKA, C. BROWN; Dept. of Biol. Sci., Boise State Univ., Boise, ID

Abstract: Parkinson's disease (PD) is the second most common neurodegenerative disease. PD is characterized by motor control deficits that arise from a loss of dopaminergic (DA) neurons in the substantia nigra (SN). However, the fundamental cause of DA neuron loss is unknown. Deficiencies in the process of adult neurogenesis have been strongly associated with Alzheimer's disease, a pathophysiologically related disorder. Conversely, investigations of neurogenesis in relation to PD have reported conflicting findings and the notion of adult neurogenesis for DA neurons remains controversial. The inability to reproduce previous studies favoring adult DA neurogenesis may result from technical limitations. To overcome these limitations, we created a mouse model to permanently label DA neuron precursors permitting mapping of their fate in adult animals. Using this model, we have discovered compelling evidence for adult DA neurogenesis. Surprisingly, we found that precursor cells responsible for mature DA neuron replacement express Nestin but not Sox2. This is the first report of a Nestin-positive/Sox2-negative neuronal progenitor pool perhaps indicating an atypical origin for these cells. Interestingly, it was also found that the rate of DA neuron regeneration mirrors that of DA neuron loss in an enhanced inflammatory response model of PD. This observation suggests that

progressive loss of DA neurons in PD might result from suppression of DA neurogenesis in adults by inflammatory response. These findings represent a substantial leap in current knowledge of adult DA neurogenesis *in vivo*, will enable improved modeling *in vitro*, and facilitate the harnessing of this process for therapeutic interventions for PD.

Disclosures: B. Morrison: None. **A. Rahman:** None. **J. Albright:** None. **I. Stojkovska:** None. **C. Brown:** None.

Poster

698. Modeling Parkinson's Disease

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Topic: C.03. Parkinson's Disease

Support: LABEX program (Université de Bordeaux)

IDEX program (Université de Bordeaux)

Title: Analysis of the extracellular matrix in a mouse model of Lewy-body induced neurodegeneration

Authors: *F. N. SORIA[1,2], B. DEHAY[1,2], E. BEZARD[1,2];
[1]Inst. Des Maladies Neurodégénératives, Bordeaux Cedex, France; [2]Univ. de Bordeaux, CNRS UMR 5293, Bordeaux, France

Abstract: The extracellular matrix (ECM) is an intricately arranged molecular framework comprised of secreted proteins and complex sugars that support cell function and survival. Changes in the highly-hygroscopic hyaluronan (HA) network, the structural scaffold of the ECM in the brain, can dramatically modify the extracellular space (ECS) volume. Parkinson´s Disease (PD) is characterized by severe neuronal loss leading to motor impairment. The neuropathological hallmark in PD is the presence of Lewy Bodies (LBs), intraneuronal proteinaceous cytoplasmic inclusions constituted, among other components, by alpha-synuclein. Abnormal alpha-synuclein species are transmitted between anatomically connected regions by a mechanism that remains unknown and the ECS appears to play a key role in the transmission of proteopathic seeds from cell to cell. Here we sought to explore the HA network in the ventral midbrain of mice injected with Lewy-body fractions derived from PD patients, an established model of alpha-synuclein-induced neurodegeneration. Since HA can be visualized with fluorescent probes, we analyzed its structure by confocal microscopy and developed a method to model and quantify the complexity of the HA network in vast regions of the brain. We provide

here a detailed description of the ECM scaffold and its spatial relationship with dopaminergic neurons, glial cells and alpha-synuclein in the substantia nigra of Lewy-body injected mice, where a substantial alteration of the ECM was observed. Our results provide insight on a crucial and underexplored component of the brain in a context of neurodegeneration and adds to our understanding of the pathophysiology of neurodegenerative disorders.

Disclosures: **F.N. Soria:** None. **B. Dehay:** None. **E. Bezard:** None.

Poster

698. Modeling Parkinson's Disease

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Program#/Poster#: 698.11/U3

Topic: C.03. Parkinson's Disease

Support: Fondation Avenir

EUrotalents/Carnot

Title: Effects of photobiomodulation by Near Infra-Red light in a mouse genetic model of Parkinson's disease

Authors: ***J. MOLET**[1], K. ARVANITAKIS[2], F. REINHART[1], C. CHABROL[1], J. LE BER[1], G. BARBOUX[1], C. PERRIN[1], M. DORCHYMONT[1], D. AGAY[1], A. BENABID[1], J. MITROFANIS[2], C. MORO[1];
[1]CLINATEC, CEA MINATEC CAMPUS, Grenoble, France; [2]Univ. of Sydney, Sydney, Australia

Abstract: Rationale: Parkinson's disease (PD) is a degenerative disorder that affects movement, due to the progressive death of dopaminergic cells in the midbrain substantia nigra pars compacta (SNc) of the basal ganglia. Unfortunately, the progression of this cell death has proven to be difficult to slow and impossible to reverse. The "gold standard" treatments for most patients with PD (dopamine replacement drug therapy with or without surgery) are effective at attenuating the motor signs, at least initially, but they do not reliably slow the progression of the disease. Thus, there is a large need for new therapeutic strategies for treatment, particularly those that offer neuroprotection against PD insult. Several studies have highlighted neuroprotective properties of photobiomodulation by near infra-red (NIR) light (low intensity light therapy) in animal models of PD induced by a neurotoxin. However, these animal models of PD, based on the acute use of neurotoxin, are not fully representative of progressive stages of the disease. Here we assessed the effect of NIR illumination in a mouse genetic model exhibiting progressive

dopaminergic cell degeneration and motor deficits, the key features of the slow progressive human PD disease.
Methods: At 6 weeks half of the Engrailed 1 heterozygous ($En1^{+/-}$) and the Wild-Type (WT) mice were treated with a twice daily NIR illumination during 90 seconds for five days per week until one year old. All mice were tested for motor activity in both open-field and running wheel tests. Dopaminergic cell degeneration was examined using tyrosine hydroxylase (TH) immunohistochemistry.
Results: With the use of our behavioural tests, we found that motor activity was not improved greatly by NIR treatment in the $En1^{+/-}$ aged mice. However, we found that NIR treatment protected many SNc dopaminergic cells from degeneration. The number of TH-immunoreactive cells was higher in $En1^{+/-}$ mice exposed to NIR light (average = ~12650 cells) compared to $En1^{+/-}$ mice not subjected to NIR treatment (average = ~10100 cells) in the SNc.
Conclusions: Although the behavioural tests and time scale we used did not reveal large improvements in motor activity, our immunohistochemical findings revealed clear evidence for neuroprotection. These data indicate that NIR can reliably slow the progression of the neurodegeneration in PD; behavioural improvements may become more obvious at later time periods or with other tests.

Disclosures: **J. Molet:** A. Employment/Salary (full or part-time): CEA/LETI/CLINATEC. 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; Fondation Avenir, Eurotalents/Carnot. **K. Arvanitakis:** A. Employment/Salary (full or part-time): University of Sydney. **F. Reinhart:** A. Employment/Salary (full or part-time): CEA. **C. Chabrol:** A. Employment/Salary (full or part-time): CEA/LETI/CLINATEC. **J. Le Ber:** A. Employment/Salary (full or part-time): CEA/LETI/CLINATEC. **G. Barboux:** A. Employment/Salary (full or part-time): CEA/LETI/CLINATEC. **C. Perrin:** A. Employment/Salary (full or part-time): CEA/LETI/CLINATEC. **M. Dorchymont:** A. Employment/Salary (full or part-time): CEA/LETI/CLINATEC. **D. Agay:** A. Employment/Salary (full or part-time): CEA/LETI/CLINATEC. **A. Benabid:** A. Employment/Salary (full or part-time): CEA/LETI/CLINATEC. **J. Mitrofanis:** A. Employment/Salary (full or part-time): University of Sydney. **C. Moro:** A. Employment/Salary (full or part-time): CEA/LETI/CLINATEC.

Poster

698. Modeling Parkinson's Disease

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Topic: C.03. Parkinson’s Disease

Support: ANR-12-BSV4-0001-01

LABEX BRAIN ANR-10-LABX-43

FP7-PEOPLE-2009-ERG256303

Fondation pour la Recherche Médicale

Fondation de France

France Parkinson Foundation

Title: Assessment of neurodegeneration and spreading of alpha-synuclein pathology induced by distinct alpha-synuclein assemblies in mice

Authors: *B. DEHAY[1], M. BOURDENX[1], L. ARCURI[1], L. BOUSSET[2], R. MELKI[2], E. BEZARD[1];
[1]Inst. of Neurodegenerative Dis., Bordeaux, France; [2]Ctr. Natl. de la Recherche Scientifique, Univ. Paris-Saclay, Paris-Saclay Inst. of Neurosci., Gif-sur-Yvette, France

Abstract: Aggregation of alpha-synuclein is implicated in several neurodegenerative diseases. Emerging evidence have strongly implicated cell-to-cell transmission of misfolded alpha-synuclein as a pathogenetic mechanism in synucleinopathies. Several experimental paradigms have been used to study alpha-synuclein propagation in animals, in particular injections of exogenous *in vitro*-generated preformed alpha-synuclein assemblies (Bousset et al., 2013) or alpha-synuclein containing Lewy Bodies (LB) extracts from *postmortem* PD patient brain tissue (Recasens et al., 2014). The aim of this study was to investigate the pathophysiological similarities and differences associated with distinct alpha-synuclein assemblies regarding toxicity and spreading in wild-type animals. We tested alpha-synuclein-containing LB extracts and two types of highly purified and structurally characterized α-syn strains denoted “fibrils” and “ribbons”, at different concentrations. Wild-type mice received a stereotactic injection in the substantia nigra of either synthetic alpha-synuclein strains or LB extracts. Four months after injection, extensive analysis was performed to assess qualitatively, quantitatively and spatially in the whole brain the extent and pattern of lesion as well as the occurrence of synucleinopathy using both biochemical and histochemical procedures. This study allows establishing whether distinct alpha-synuclein assemblies follow different propagation pathways and exhibit specific tropism for cells or circuits. The underlying idea was to address key questions common to synucleinopathies, and develop a reliable model for testing neuroprotective therapies.

Disclosures: **B. Dehay:** None. **M. Bourdenx:** None. **L. Arcuri:** None. **L. Bousset:** None. **R. Melki:** None. **E. Bezard:** None.

Poster

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Topic: C.03. Parkinson's Disease

Support: national natural science foundation of china (81371398)

Natural Science Foundation of Beijing (7131001)

Title: The mechanism of rotenone induces alpha-synuclein phosphorylation by reducing PP2A activity

Authors: *H. YANG[1,2];
[1]Neurobio., Parkinson's Dis. Center, Capital Med. Univer, Beijing City, China; [2]Beijing Inst. for Brain Disorders, Beijing, China

Abstract: Rotenone has been shown to induce many parkinsonian features and has been widely used in chemical models of Parkinson's disease (PD). Its use is closely associated with α-synuclein (α-syn) phosphorylation both *in vivo* and *in vitro*. However, the mechanisms whereby rotenone regulates α-syn phosphorylation remain unknown. Protein phosphatase 2A (PP2A) has been shown to play an important role in α-syn dephosphorylation. We therefore investigated if rotenone caused α-syn phosphorylation by down-regulation of PP2A activity in mice. Rotenone increased the phosphorylation of α-syn at Ser129, consistent with the inhibition of PP2A activity by increased phosphorylation of tyrosine 307 of PP2A catalytic (pTyr307 PP2Ac). We further explored the interactions among rotenone, PP2A, and α-syn in SK-N-SH cells and primary rat cortical neurons. Rotenone inhibited PP2A activity via phosphorylation of PP2Ac at Tyr307. The reduction in PP2A activity and rotenone cytotoxicity were reversed by treatment with the PP2A agonist, C2-ceramide, and the SRC kinase inhibitor, SKI606. Immunoprecipitation experiments showed that rotenone induced an increase in calmodulin-SRC complex in SK-N-SH cells, thus activating SRC kinases, which in turn phosphorylated PP2A at Tyr307 and inhibited its activity. C2-ceramide and SKI606 significantly reversed the rotenone-induced phosphorylation and aggregation of α-syn by increasing PP2A activity. These results demonstrate that rotenone-reduced PP2A activity via SRC kinases is involved in the phosphorylation of α-syn. These findings clarify the novel mechanisms whereby rotenone can induce PD.

Disclosures: H. Yang: None.

Poster

698. Modeling Parkinson's Disease

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Michael J. Fox Foundation, Research Grant

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Title: Evidence for an inflammatory trigger leading to exacerbated neuroinflammation in the G2019S LRRK2 rat model - a longitudinal *In vivo* study

Authors: A. SCHILDT[1,5], M. D. WALKER[1], K. DINELLE[1], R. KORNELSEN[1], M. MEJIAS[2], Q. MIAO[6], C. TAKHAR[6], Y. OBAYASHI[2], N. HOSSAIN[2], J. O'KUSKY[3], M. FARRER[4], *D. J. DOUDET[7,2], V. SOSSI[1];
[1]Dept. of Physics and Astronomy, [2]Dept. of Medicine, Neurol., [3]Dept. of Pathology and Lab. Med., [4]Dept. of Med. Genet., Univ. of British Columbia, Vancouver, BC, Canada; [5]Dept. of Nuclear Med. and Mol. Imaging, Univ. Med. Ctr. Groningen, Univ. of Groningen, Groningen, Netherlands; [6]TRIUMF, Vancouver, BC, Canada; [7]Univ. British Columbia, Vancouver, BC, Canada

Abstract: Mutations of the leucine rich repeat kinase (LRRK2) are the most common genetic risk factor for Parkinson's disease (PD). Although the pathogenic mechanisms by which LRRK2 mutations facilitate the development of PD remain largely unknown, LRRK2 has been implicated in neuroinflammation. We investigated if LRRK2 mutations exacerbate neuroinflammation in response to an inflammatory trigger using lipopolysaccharide (LPS) and, consequently may lead to selective degeneration of dopaminergic neurons.
Rats carrying the G2019S LRRK2 mutation and wild type (WT) litter mates were subjected to an acute inflammatory insult using a 3 mg/kg LPS dose injected intraperitoneally (i.p.) at 4 months of age. A longitudinal PET study using ^{11}C-PBR28, a marker of microglia activation, ^{11}C-DTBZ, a VMAT2 marker of dopaminergic integrity and ^{18}F-DOPA, a marker of dopamine synthesis and turnover, was performed at baseline and 1 week to 10 months post-LPS. Behavioral tests were performed 6-10 months after treatment. Postmortem immunohistochemistry (IHC) for dopaminergic (tyrosine hydroxylase, TH) and inflammatory (CD68) markers is being performed. No difference in any of the PET markers was observed at baseline. The longitudinal ^{11}C-PBR, corrected for ageing, showed significant time effects between baseline and 10 months ($p = 0.035$). Additionally, a highly significant difference between the saline and LRRK2-LPS group was observed at 10 months post-LPS ($p =< 0.001$). No selective degeneration of dopaminergic

terminals or in dopamine synthesis and turnover was observed in TG or WT rats with ^{11}C-DTBZ and ^{18}F-DOPA. No treatment genotype interactions were observed with behavioural tests. TG animals showed a deficit in the rotarod performance independent of treatment. LPS treated animals showed decreased number of sniffs in olfactory test ($p = 0.04$ (TG), $p = 0.002$ (WT)). Preliminary analysis of the postmortem TH stain revealed no changes in density of dopaminergic neurons matching our ^{11}C-DTBZ PET results.
This longitudinal in vivo study supports the hypothesis that G2019S LRRK2 mutations increase neuroinflammation following an acute inflammatory insult. However, our PET and preliminary IHC results do not show selective degeneration of the dopaminergic system by a single acute exposure to an inflammatory trigger as shown in other models of PD. Currently, we are completing the postmortem analysis by IHC to validate our PET results.
The effect of repeated rather than single insults should be explored in further studies as well as the effect of inflammatory triggers on other systems than the dopaminergic system.

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Poster

698. Modeling Parkinson's Disease

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Topic: C.03. Parkinson’s Disease

Support: Interdisziplinäres Zentrum für Klinische Forschung (IZKF)-ProjektnummerA-303rd

Title: Lymphocytes contribute to motor deficits in the AAV1/2 A53T alpha-synuclein mouse model of Parkinson’s disease

Authors: ***A. A. KARIKARI**[1], M. GEHMEYR[1], E. RIBECHINI[2], V. MALTESE[1], S. KNORR[1], J. VOLKMANN[1], J. M. BROTCHIE[3], J. B. KOPRICH[3], M. B. LUTZ[2], C. W. IP[1]; [1]Dept. of Neurology, Universitätsklinikum Würzburg, Wuerzburg, Germany; [2]Inst. für Virologie und Immunbiologie, Univ. Würzburg, Würzburg, Germany; [3]Toronto Western Res. Inst., Toronto, ON, Canada

Abstract: Background: The role of the adaptive immune system in the progression of Parkinson’s disease is still elusive. While changes have been described in immune response during PD, it is not evident if these alterations are pathogenically relevant. Results from animal models of PD have been controversial with reports of detrimental effect of T-cells in the MPTP

mouse model and a protective role in the 6-OHDA model. In this study, we characterized the impact of the adaptive immune system in a novel, non-toxic PD model. The model of AAV1/2 mediated overexpression of human A53T α-synuclein (aSyn) was chosen because it better reflects the clinical and paraclinical aspects of the human disease than the mentioned toxin models.
Objective: To ascertain the contribution of the adaptive immune system in the pathogenesis of the AAV1/2 driven expression of human A53T aSyn model of PD.
Methods: AAV1/2 A53T aSyn or AAV1/2 empty vector (EV) at a concentration of 5.1 x 10exp12 gp/ml were unilaterally injected into the right substantia nigra (SN) of male adult C57BL/6 or RAG-1-/- mice lacking mature T-and B-lymphocytes. Additionally, another group of RAG-1-/- mice that received wildtype bone marrow (RAG-1-/- wt BM AAV1/2 A53T aSyn), was included into the study. Clinical examination was performed by rotarod analysis and cylinder test examining paw use asymmetry. Immunohistochemistry was used to determine lymphocyte infiltration in the SN. FACS analysis was implemented to investigate brain infiltrates of immune cells.
Results: Significant impairment of rotarod performance and paw use asymmetry in the cylinder test with preference for the right front paw was observed in the wt A53T aSyn injected group compared to the wt EV group at 8 weeks post injection (rotarod $p<0.05$; cylinder test $p<0.01$). Immune deficient RAG-1-/- A53T aSyn injected mice improved significantly in their motor assessments compared to immune competent wt A53T aSyn mice (rotarod $p<0.05$; cylinder test $p<0.001$). RAG-1-/- wt BM A53T aSyn mice however demonstrated a significant deterioration in motor behavior compared to immune deficient RAG-1-/- A53T aSyn mice (rotarod and cylinder test $p<0.05$). Immunohistochemical staining revealed an increase of CD8>CD4 T-cell numbers in wt A53T aSyn SN. FACS analysis using CD69 as activation marker showed a high activation and infiltration of CD8+ and CD4+ T-cells in the brain after A53T aSyn injection.
Conclusions: The presence of lymphocytes worsens clinical symptoms in this AAV1/2 A53T aSyn mouse model of PD.

Disclosures: **A.A. Karikari:** None. **M. Gehmeyr:** None. **E. Ribechini:** None. **V. Maltese:** None. **S. Knorr:** None. **J. Volkmann:** None. **J.M. Brotchie:** None. **J.B. Koprich:** None. **M.B. Lutz:** None. **C.W. Ip:** None.

Poster

698. Modeling Parkinson's Disease

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Topic: C.03. Parkinson’s Disease

Support: Alpha-synuclein and GFP viral vectors were kindly obtained from Michael J Fox Foundation.

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Title: Hippocampal adeno-associated viral vector-mediated alpha-synuclein overexpression: a promising model for recapitulating cognitive involvement in Parkinson's disease

Authors: *E. CINAR[1,2], G. YALCIN-CAKMAKLI[3], S. U. MUTLUAY[2], E. SAKA[4], A. ULUSOY[5], B. C. TEL[2], B. ELIBOL[4];
[1]INSTITUTION OF HEALTH SCIENCES, Ankara, Turkey; [2]Dept. of Pharmacol., [3]Inst. of Neurolog. Sci. and Psychiatry, [4]Dept. of Neurol., Hacettepe Univ., Ankara, Turkey; [5]German Ctr. for Neurodegenerative Dis. (DZNE), Bonn, Germany

Abstract: Aim: In this study, our goal is to induce cognitive and motor dysfunction in an animal model aimed to recapitulate the late stages of Parkinson's disease (PD) dementia by adeno-associated viral vector (AAV)-mediated alpha-synuclein (a-syn) overexpression bilaterally in dentate gyrus (DG) together with substantia nigra (SN). Methods: Female Sprague-Dawley rats (200-250g) were used for streotactic injections. AAV-carrying either a-syn (n=11) or green fluorescent protein (GFP; n=11) gene, or as a sham control just saline (n=8) injected bilaterally both into DG and SN. Further seven animals used as naïve control. All animals were tested for memory, spatial learning, anxiety, hedony, motor coordination and locomotion by novel object recognition (NOR), Morris water maze (MWM), elevated plus maze (EPM), sucrose preference, rotarod tests and basal and apomorphine induced locomotor activity test respectivelty, between 16-18 weeks following injection. Brain samples were analyzed by western blotting for semi-quantitative a-syn and GFP expression. The study was approved by Hacettepe University Animal Experimentations Local Ethics Board (2014/51-08). Results: A-syn and GFP expression was shown both in DG and SN by western blotting. A-syn group spent shorter time with the novel object in NOR test, spent longer time to find the platform in MWM and spent longer time on open arm in EPM compared to controls ($p<0,05$). Also a-syn group consumed less sucrose compared to controls ($p<0,05$). In rotarod test; a-syn group displayed worse motor performance on the rod compared to controls ($p<0,05$). In locomotor activity test; all the groups showed increase in horizontal activity after apomorphine injection compare to basal activity however, only a-syn group showed a marked increase compare to controls ($p<0,05$). Conclusion: A-syn overexpression in SN and DG together led to memory impairment, spatial learning deficits and anhedonia besides motor dysfunction and apomorphine sensitivity. Neuronal loss as well as preceeding synaptic dysfunction in hippocampus together with SN might be responsible for the cognitive and motor impairments. The underlying mechanisms of these changes will be examined with further histopathological analysis.

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Poster

698. Modeling Parkinson's Disease

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Topic: C.03. Parkinson's Disease

Title: Histological evaluation of synaptophysin and spinophilin in parkinson's disease animal models

Authors: *B. HUTTER-PAIER[1], J. WIESER[2], J. NEDDENS[1], E. AUER[1];
[1]Neuropharm., QPS Austria GmbH, Grambach, Austria; [2]Biomed. Sci., Univ. of Applied Sci., Graz, Austria

Abstract: PD is a progressive movement disorder of the nervous system, which is characterized by motor-symptoms like tremor, rigidity and bradykinesia. Synapse degeneration of neurons in PD-relevant brain regions is typical for PD patients that develop dementia and was recently shown to be related to synaptic alpha-synuclein accumulation and aggregation. Alpha-synuclein is a presynaptic neuronal protein and is linked to the pathogenesis of PD. Only little is known about synapse loss and spine densities in PD mouse models that should mimic the human disease. To elucidate the quantitative distribution of synapses and spine density in the hippocampus of Line 61, D-Line and A53T transgenic mice, synaptophysin and spinophilin levels were analyzed over age. Synaptophysin is a vesicle protein and is presynaptically expressed while spinophilin is a postsynaptic marker highly enriched in dendritic spines. Our results show that in Line 61 transgenic mice both markers are highly increased at 6 months but not in other age groups. In D-Line mice, both markers are significantly decreased in all age groups while in A53T transgenic mice none of the markers is altered. Our results show different effects of alpha-synuclein expression on synaptic density and dendritic spines in the hippocampus of three different transgenic mouse models.

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Poster

698. Modeling Parkinson's Disease

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Topic: C.03. Parkinson's Disease

Support: Merck Serono

Title: Microglial activation in mice with glucocerebrosidase deficiency in dopaminergic neurons

Authors: *S. DOVERO[1], F. SORIA[1], M. ENGELN[1], B. DEHAY[1], E. NORMAD[2], F. GEORGES[1], C. LO BIANCO[3], E. BEZARD[1], P.-O. FERNAGUT[1];
[1]Inst. of Neurodegenerative Dis., Bordeaux, France; [2]Interdisciplinary Inst. of Neurosciences, UMR 5297, Bordeaux, France; [3]: Diagnos. Develop. Services, Covance Central Lab. Services, Geneva, Switzerland

Abstract: Heterozygous mutations in the gene encoding the lysosomal hydrolase beta-glucocerebrosidase (GBA1) are an important risk factor for Parkinson's disease (PD). *In vitro*, altered GBA activity promotes alpha-synuclein accumulation and elevated levels of alpha-synuclein compromise GBA enzymatic function, thus supporting a pathogenic mechanism in PD. However, the mechanism by which GBA deficiency is linked to increased risk of PD *in vivo* remains unknown. Since knocking-out GBA in the entire central nervous system (CNS) in mouse induces massive neurodegeneration associated with early death, we sought to generate a mouse model of dopaminergic GBA deficiency to investigate the long-term consequences of compromised GBA function. We bred DAT-Cre with GBA-floxed mice to obtain selective homozygous disruption of glucocerebrosidase in midbrain dopamine neurons (DAT-GBA-KO), and analysed motor function, neuronal survival, alpha-synuclein phosphorylation and glial activation, in aged animals as well as in mice overexpressing the mutant (A53T) form of adenoviral-delivered human alpha-synuclein. Our results show that although DAT-GBA-KO mice display increased microglial activation in the substantia nigra, there is no neurodegeneration and alpha-synuclein pathology with respect to wild-type mice. This raises the question whether neuronal GBA deficiency is important for PD pathogenesis and suggests the involvement of GBA deficiency in other cell types as a potential mechanism.

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Poster

698. Modeling Parkinson's Disease

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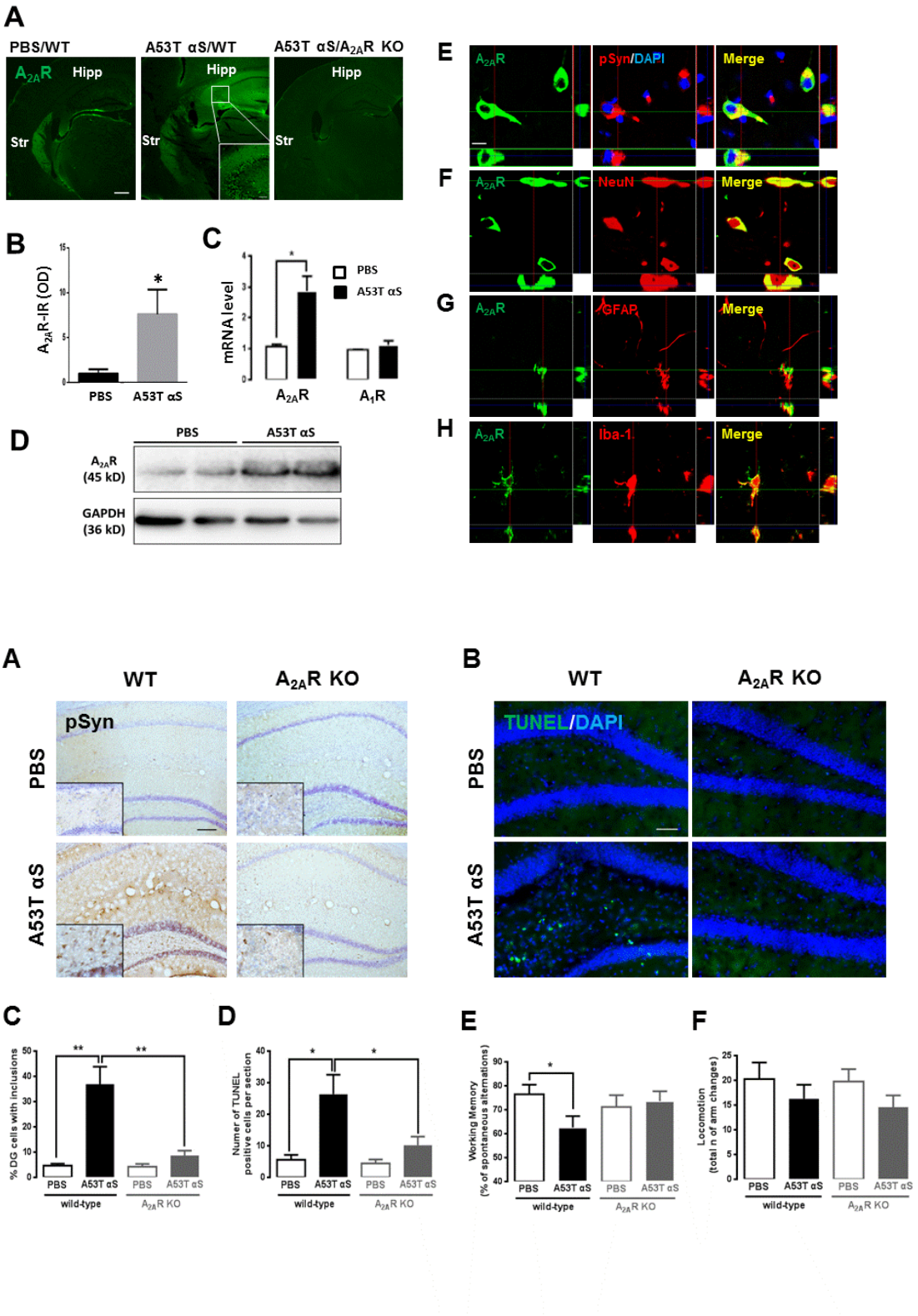
Title: Aberrant adenosine A_{2A} receptor signaling contributes to neurodegeneration and cognitive impairments in a mouse model of synucleinopathy

Authors: *R. XIANGPENG[1], H. QIDI[1], L. YA[1], C. JIANG-FAN[1,2];
[1]The Eye Hosp. of Wenzhou Med. Univ., Zhejiang, China; [2]Dept. of Neurol., Boston University, Sch. of Med., Boston, MA

Abstract: Synucleinopathy is characterized by abnormal accumulation of misfolded α-synuclein (α-Syn)-positive cytoplasmic inclusions and by neurodegeneration and cognitive impairments, but the pathogenesis mechanism of synucleinopathy remains to be defined. Using a transmission model of synucleinopathy by intracerebral injection of preformed A53T α-Syn fibrils, we investigated whether aberrant adenosine A_{2A} receptor ($A_{2A}R$) signaling contributed to pathogenesis of synucleinopathy. We demonstrated that intra-hippocampal injection of preformed mutant α-Syn fibrils triggered a striking and selective induction of $A_{2A}R$ expression which was closely co-localized with pSer129 α-Syn-rich inclusions in neurons and glial cells of hippocampus. Importantly, by abolishing aberrant $A_{2A}R$ signaling triggered by mutant α-Syn, genetic deletion of $A_{2A}Rs$ blunted a cascade of pathological events leading to synucleinopathy, including pSer129 α-Syn-rich and p62-positive aggregates, NF-κB activation and astrogliosis, apoptotic neuronal cell death and working memory deficits without affecting motor activity. These findings define α-Syn-triggered aberrant $A_{2A}R$ signaling as a critical pathogenesis mechanism of synucleinopathy with dual controls of cognition and neurodegeneration by modulating α-Syn aggregates. Thus, aberrant $A_{2A}R$ signaling represents a useful biomarker as well as a therapeutic target of synucleinopathy.



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Poster

698. Modeling Parkinson's Disease

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Topic: C.03. Parkinson's Disease

Support: DOD W81XVVH-12-1-0051

Title: Viral vector mediated- human alpha-synuclein overexpression induces early axonopathy in the mouse nigro-striatal dopaminergic system.

Authors: *A. P. TAGLIAFERRO, T. KAREVA, N. KHOLODILOV, R. BURKE;
Columbia Univ., New York, NY

Abstract: Alpha-synuclein (α-syn) is the major component of Lewy bodies, histopathological hallmark of Parkinson's disease (PD), and it is implicated in both sporadic and familial cases. Point mutations (A30P, E46K, A53T) as well as whole locus multiplications in the α-syn gene cause autosomal dominant PD (Hardy et al., 2006). In this study we investigated the effects of human wild type α-syn overexpression in the mouse nigrostriatal dopaminergic system using an adeno-associated viral vector (AAV) construct which uses the neuron-specific synapsin-1 promoter. Adult mice (2 months old) received unilateral intranigral injection of either AAV2/7-α-syn or AAV2/7-tomato (used as a control protein) and they were sacrificed 4 and 8 weeks after injection. Tyrosine hydroxylase (TH) immunostaining was used to investigate the number of TH-positive neurons in the substantia nigra (SN), level of TH expression in the striatum and the number of dopaminergic axons in the medial forebrain bundle (MFB). In addition, TH-positive axons in the MFB were examined for signs of axonopathy, Including spheroids and fragmentation. At 4 weeks, experimental animals showed the presence of TH-positive axonal spheroids in the MFB while there was no decrease in the number of TH-positive neurons in the SN or TH-positive axons in the MFB. However, the level of TH expression in the dorso-lateral striatum was decreased. At 8 weeks α-syn overexpression induced a decrease in the number TH-positive neurons in the posterior region of the SN, the levels of TH expression in the dorso-lateral striatum and the number of TH-positive axons in the MFB. Axonal spheroids continued to be present in the MFB. The time course of changes observed in this mouse model of viral vector mediated human α-syn overexpression shows that an axonopathy is the first sign of pathology of the nigro-striatal dopaminergic system. This model will provide an opportunity to investigate the mechanisms involved in early axonal degeneration as well as new therapeutic approaches aimed to restore dopaminergic innervation.

Disclosures: A.P. Tagliaferro: None. **T. Kareva:** None. **N. Kholodilov:** None. **R. Burke:** None.

Poster

698. Modeling Parkinson's Disease

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Program#/Poster#: 698.21/U13

Topic: C.03. Parkinson's Disease

Title: Novel mouse model for the initiation and propagation of alpha-synuclein oligomerization in Parkinson's disease

Authors: K. MARTIN[1], B. VON EINEM[1], V. GROZDANOV[1], C. BLIEDERHAEUSER[1], D. MARKX[2], J. WEISHAUPT[1], K. DANZER[1];
[1]Experimentelle Neurologie, Universitätsklinikum Ulm, Ulm, Germany; [2]Inst. für Proteinbiochemie, Univ. Ulm, Ulm, Germany

Abstract: Emerging evidence suggests that in contrast to Lewy bodies, alpha-synuclein (α-syn) oligomers are the principle toxic species in Parkinson's disease (PD). The concept of α-syn oligomers being transmitted from cell-to-cell in a prion-like manner is a subject of current debate and suitable *in vivo* models testing this hypothesis are largely missing. We have therefore generated two novel neuronal human α-syn overexpression mouse models in a tet-off-system inducible manner, based upon a reporter protein-fragment complementation assay. In our mouse lines, α-syn is fused to the N-terminal half of either human Gaussia Luciferase (S1) or venusYFP (V1S) and α-syn is fused to C-terminal half of human Gaussia luciferase (S2) or venusYFP (SV2). When α-syn starts to oligomerize, the reporter fragments will be in close proximity, complementing to an active enzyme or fluorescent protein.
Our results indicate a successful α-syn oligomer formation *in vivo* and that a variety of different α-syn oligomer species are present in different mouse brain areas as demonstrated by western blot analysis, immunofluorescent staining, size-exclusion chromatography and subsequent luciferase measurements.
Employing an inducible transgenic α-syn oligomer CaMKIIα mouse model allows spatial expression of α-syn oligomers restricted to specific brain regions. Thus spreading of viral particles in viral vector-mediated α-syn overexpression models or transmission of recombinant proteins due to blood stream transport in models of intracerebral inoculation of recombinant oligomers can be avoided. Neurons overexpressing the α-syn transgene under control of the CaMKIIα promoter transport α-syn to the pre-synaptic compartment. In contrast, neurons without CaMKIIα promoter activity lack pre-synaptic human α-syn oligomers but reveal only cytoplasmic aggregates of α-syn oligomers, indicating a neuron-to-neuron transmission of oligomers. Most importantly, spreading of α-syn-oligomers from neurons to other cell types in the brain like microglia or oligodendrocytes was also observed, possibly concomitant with secondary deleterious effects.
The formation of α-syn oligomers in our mouse models is accompanied by changes in animal

behavior in aged mice over 12 months of age. Significant motor deficits emerged as the major phenotype, regarding bradykinesia, fine motor coordination, balance and grip strength, analyzed by Pole-test, beam walking and accelerating RotaRod.
In summary, our new inducible transgenic α-syn oligomer mouse model provides a suitable tool for studying α-syn oligomerization, spreading and toxicity *in vivo*.

Disclosures: K. Martin: None. **B. von Einem:** None. **V. Grozdanov:** None. **C. Bliederhaeuser:** None. **D. Markx:** None. **J. Weishaupt:** None. **K. Danzer:** None.

Poster

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Topic: C.03. Parkinson’s Disease

Support: NIH Grant R01NS082565

Title: Analysis of alpha-synuclein pathology in PINK1 knockout rats.

Authors: *R. B. CREED, M. S. GOLDBERG;
Ctr. for Neurodegeneration and Exptl. Therapeutics, Dept. of Neurol, Univ. of Alabama At Birmingham, Birmingham, AL

Abstract: Mutations in the PTEN induced kinase 1 (PINK1) gene cause autosomal recessive Parkinson’s disease (PD). The main pathological hallmarks of PD are loss dopamine neurons in the substantia nigra pars compacta, which are required for normal movement, and the formation of alpha-synuclein rich aggregates termed Lewy body inclusions. Previous studies of PINK1 knockout (KO) rats have reported mitochondrial dysfunction, behavioral deficits, loss of neurons in the substantia nigra and locus coeruleus, and alpha-synuclein aggregates in various brain regions. We sought to characterize PINK1 KO rats specifically with respect to alpha-synuclein pathology because spontaneous formation of alpha-synuclein aggregates (without alpha-synuclein overexpression or injection) is a rare and important feature of PD animal models and because abnormal alpha-synuclein has been implicated both genetically and neuropathologically as a key mechanism of PD pathogenesis. Given PINK1’s proposed function in mitochondrial autophagy, we also investigated the abundance of key mitochondrial proteins in the brains of PINK1 KO rats. We observed alpha-synuclein-immunoreactive aggregates in various brain regions of PINK1 KO rats including cortex, thalamus, striatum and ventral midbrain, but nowhere in wild-type (WT) rats. Proteinase K treatment revealed protease-resistant alpha-synuclein in the brains of PINK1 KO rats, however, the inclusions themselves were not

proteinase K resistant. Co-imunofluorescence showed that the alpha-synuclein-immunoreactive aggregates are both ubiquitin immunoreactive and thioflavin S positive. We did not find any tau-immunoreactive pathology or any differences between WT and KO rats in markers of neuroinflammation, such as GFAP and Iba1. Western analysis showed similar levels of key mitochondrial proteins in the brains of WT and PINK1 KO rats. Together, this data indicates that PINK1 deficiency can directly lead to abnormal alpha-synuclein aggregation in vivo. This suggests that alpha-synuclein aggregation may be directly involved in PD-related neurodegeneration caused by PINK1 mutations.

Disclosures: R.B. Creed: None. **M.S. Goldberg:** None.

Poster

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Topic: C.03. Parkinson’s Disease

Support: H-1301

Title: Dopamine neuron specific translational profiling in a transgenic mouse model of PD

Authors: *K. WAGNER[1,2], S. JANEZIC[1], F. WESSELY[1], J. MONZON SANDOVAL[1,2], C. WEBBER[1,2], R. WADE-MARTINS[1,2];
[1]Dept. of Physiology, Anat. and Genet. (DPAG), Univ. of Oxford, Oxford, United Kingdom; [2]Oxford Parkinson's Dis. Ctr. (OPDC), Oxford, United Kingdom

Abstract: Aims:
To characterise and investigate targeted cell-type specific gene expression in a *TH-bacTRAP* transgenic mouse model.
Methods:
Early translational changes are examined specifically in dopaminergic neurons during Parkinson's disease (PD) *in vivo* by translating ribosome affinity purification (TRAP) in BAC-transgenic mice. These mice express an eGFP-tagged ribosomal protein (eGFP-L10a) under the control of the cell-type specific promoter of tyrosine hydroxylase (TH) specifically in dopaminergic neurons.
Transgene expression was characterized by native eGFP fluorescence and double immunohistochemistry for TH/GFP. Translated mRNAs have been immunoprecipitated from midbrains of homozygous *TH-bacTRAP* transgenic mice and littermate controls. The isolated mRNA has been analysed by qRT-PCR before performing gene expression profiling by

RNAseq.
Results:
The molecular characterisation of *TH-bacTRAP* mice reveals expression of eGFP in TH+ dopaminergic neurons. Pilot affinity-purifications of eGFP-tagged ribosomes and bound mRNAs from this defined cell population has been performed. The analysis of immunoprecipitated mRNA vs. total RNA via qRT-PCR and RNA-Seq demonstrate the specific enrichment of dopaminergic markers in *TH-bacTRAP* mice after TRAP.
Conclusion:
We could show that high-quality RNA can be isolated specifically from dopaminergic neurons. Next, we will combine TRAP methodology with our mouse model of PD that overexpresses alpha-synuclein from the complete human *SNCA* locus. Comparing our PD model with healthy controls will allow us to investigate perturbations in the expression of translated genes early during disease progression.

Disclosures: K. Wagner: None. **S. Janezic:** None. **F. Wessely:** None. **J. Monzon Sandoval:** None. **C. Webber:** None. **R. Wade-Martins:** None.

Poster

698. Modeling Parkinson's Disease

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Support: MSU Vice President for Research and Graduate Studies

The Udall Center of Excellence at Michigan State University NS058830

Edwin Brophy Endowment in Central Nervous System Disorders

Title: CRISPR-Cas9 mediated knock-out of Parkinson's disease related genes in that rat substantial nigra *In vivo*

Authors: *I. M. SANDOVAL[1], R. C. SELLNOW[1], B. DALEY[1], N. KUHN[1], N. MARCKINI[1], A. C. STRAUSS[1], J. W. LIPTON[1,2], F. P. MANFREDSSON[1,2], T. J. COLLIER[1,2];
[1]Translational Sci. and Mol. Med., Michigan State Univ. Clin. and Translational Sci. Inst., Grand Rapids, MI; [2]Mercy Hlth. Hauenstein Neurosci. Ctr., Grand Rapids, MI

Abstract: The ability to knock out a desired gene in a specific cell type enables direct probing of gene function. The newest class of nucleases, CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9 system, can be used to introduce frame-shifting mutations to knock

out a desired gene with high precision. It has been widely used in a variety of cells and microorganisms, but its application in mature neuronal tissue has been limited. We sought to investigate whether we could achieve efficient CRISPR/Cas9-mediated gene inactivation *in vivo*, specifically in dopaminergic neurons of the substantia nigra, the cells affected in Parkinson's disease (PD). As a proof of concept, we selected to target tyrosine hydroxylase (TH), the rate limiting enzyme in the production of the neurotransmitter dopamine. We also targeted alpha-synuclein (α-syn), vesicular transport protein 35 (VPS35) and EIF4G1, all of which are linked to familial forms of PD. We designed guide RNAs (gRNAs) against exon 1 and exon 2 of the TH gene and confirmed genomic DNA cleavage efficiency and reduction in protein levels *in vitro* in PC12 cells. We delivered the protein Cas9, together with the gRNAs as well as GFP as a transduction marker into the substantia nigra of 2 month old Sprague-Dawley rats using recombinant adeno-associated viral vectors (rAAVs). Immunostaining of brain sections revealed a robust decrease of TH protein expression in CRISPR/gRNA(TH) treated brains as compared to "unguided" control injected rats six weeks after surgery . Stereological quantification of TH immunoreactive neurons revealed a 48% reduction of TH expressing nigral neurons in the treated animals. These results validate the use of CRISPR technology *in vivo* in the nigrostriatal dopaminergic system in mature brains. Current experiments are focused to target the aforementioned PD-related genes: α-syn, VPS35 and EIF4G1 using a similar strategy. Upon project completion we will have a useful tool-box to interrogate gene function in nigral dopamine neurons *in vivo* while still in their native context

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Poster

698. Modeling Parkinson's Disease

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Topic: C.03. Parkinson's Disease

Title: Characterization of a novel conditional alpha-synuclein knockout mouse model

Authors: *K. MILLER[1,2], I. M. SANDOVAL[1,3], M. J. BENSKEY[1], C. E. SORTWELL[1,2,3], T. J. COLLIER[1,2,3], J. W. LIPTON[1,2,3], F. P. MANFREDSSON[1,2,3];
[1]Translational Sci. and Mol. Med., Michigan State Univ., Grand Rapids, MI; [2]Neurosci. Program, Michigan State Univ., East Lansing, MI; [3]Mercy Hlth. St. Mary's Hauenstein Neurosci. Ctr., Grand Rapids, MI

Abstract: The pathological hallmarks of Parkinson's disease (PD) are Lewy bodies and neurodegeneration of dopamine (DA) neurons of the substantia nigra pars compacta (SNc). The protein alpha-synuclein (α-syn) is the primary component of Lewy bodies and mutations in the α-syn gene result in familial forms of PD. Moreover, overexpression of α-syn results in aggregation and neurodegeneration. Consequently, the predominant hypothesis in PD posits that α-syn aggregates, or the process of aggregation, is a directly toxic event in PD pathology. The positive association between α-syn aggregation and PD progression has made α-syn a prevalent target for therapeutic strategies aimed at decreasing α-syn from affected neurons. However, work from our lab and others has demonstrated that removal of α-syn in mature DA neurons of mice, rats, and non-human primates result in dose-dependent and progressive neurodegeneration. Furthermore, this cell loss can be rescued by supplementation of α-syn. These results demonstrate that α-syn is essential for the survival of mature DA neurons. This is in stark contrast to the germline α-syn knockout, which does not show any overt neurodegeneration. To better study the consequences following α-syn removal in mature neurons *in vivo* we generated a conditional knockout mouse. Mice were engineered to have Exons 1-2 of the α-syn gene flanked by loxP sites. Successful insertion of LoxP sites was confirmed via genomic analysis and sequencing. Importantly, the inclusion of a LoxP site in the 5'UTR of the SNCA gene did not influence α-syn expression as confirmed via Western blot. Finally, we confirmed that expression of CRE recombinase (iCRE) excises the floxed genomic region, eliminating α-syn protein expression. To provide spatio-temporal control of α-syn removal, we utilized recombinant adeno-associated virus (rAAV) to deliver iCRE to the SNc. The integrity of the nigrostriatal system will be assessed at 1, 2, 4 and 6 months post-surgery via behavioral analysis. SNc neuron loss will be quantified using stereological cell counting of tyrosine hydroxylase and NeuN positive cells, and levels of striatal DA will be quantified using HPLC. We predict that a complete loss of α-syn (homozygous floxed) will result in more severe neurodegeneration and motor phenotype than partial loss of α-syn (heterozygous floxed). Once validated, this new model will provide a foundation for investigations into the physiological function of α-syn without the potential confounds that may occur due to genetic compensations following α-syn removal during development. Importantly, this model will also be valuable when investigating the role of α-syn in PD etiology.

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Poster

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Topic: C.03. Parkinson's Disease

Support: Luxembourg FNR CORE Grant PDDJ1Neurodeg

Title: Profound shifts of metabolic profile accompany neurodegeneration in murine models of parkinson's disease.

Authors: ***M. J. BUTTINI**[1], F. GIESERT[2], P. GARCIA[1], P. DIRSCHERL[2], C. JAEGER[1], D. TRUEMBACH[2], E. GLAAB[1], A. ULUSOY[3], D. VOGT-WEISENHORN[2], A. ZIMPRICH[2], R. BALLING[1], D. DIMONTE[3], W. WURST[2];
[1]Luxembourg Ctr. For Systems Biomedicine, Esch-sur-Alzette, Luxembourg; [2]Developmental Genet., Helmholtz Ctr., Munich, Germany; [3]German Ctr. for Neurodegenerative Dis., Bonn, Germany

Abstract: Understanding early, and therefore often subtle, disease processes in Parkinson's disease (PD) is essential for the development of disease modifying cures. But in patients, and in many PD animal models, measurable neurological symptoms typically occur at disease stages in which neuronal injury and loss has already progressed beyond repair. To investigate if measures of metabolite changes can help detect early disease processes, and thus open the way to biomarker and/or target identification, we investigated metabolic profiles in the brain of various genetic and induced PD models. Different aspects and disease stages of PD can be modeled in rodents by genetic (transgenic, knock-in or -out) of PD-associated genes, or by inducing spreading of misfolded alpha-synuclein (aSyn) through administration of an aSyn seeding source. We analyzed genetic models (LRRK1 knockin, SNCA transgenic, DJ-1 knockout, and crosses thereof) and induced models (spreading of aSyn induced by intrastriatal injection of recombinant aSyn fibrils in mice, or by intravagal injection of a construct expressing human aSyn in rats). We investigated metabolic profiles in brain extracts of these models using a Gas-Chromatography/Mass-Spectrometry platform, and compared them with behavioral and neuropathological measures. We analyzed changes in individual metabolites, as well as, using Principal Component Analysis or supervised machine learning, shifts in metabolite populations and the known cellular pathways they are associated with. By analyzing the brains of these models at different ages or disease stages, we drew out metabolic shifts that coincided with subtle, early motor disturbances (genetic models), and were, for the most part, independent of neuropathological changes such as the presence of aSyn aggregates, and overt neurodegeneration (induced models). Some metabolites, many them still unknown, correlated with motor performance in genetic models. Thus, by applying metabolic profiling in PD rodent models, we identified novel disease stage-related changes in metabolites, and these observations could pave the way for a better understanding of early PD disease processes.

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Poster

698. Modeling Parkinson's Disease

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Topic: C.03. Parkinson's Disease

Title: Comparison of transgenic mouse models of Parkinson's disease

Authors: M. SASNER[1], R. Y. WILPAN[1], R. P. LYNCH[1], A. K. MARTIG[2], T. MARTINEZ[2], *S. PADMANABHAN[2], K. DAVE[2];
[1]The Jackson Lab., Bar Harbor, ME; [2]The Michael J. Fox Fndn., New York, NY

Abstract: Animal models of Parkinson's disease (PD) are critical to the development of novel therapeutics. While there are a wide variety of existing mouse models of PD, none are ideal for therapy development projects, many are not available for for-profit use, and there has been no rigorous comparison of their molecular and phenotypic characteristics. The overall goals of this effort are to develop, characterize, and make available animal models of PD for both basic research and therapy development. The existing repository collection includes transgenic models over-expressing human *SNCA* with the E46K, A53T, or A30P patient mutations as well as a *GBA k*nock-in D409V allele. By combining a transgenic model over-expressing wild-type human *SNCA* with the *GBA* mutant, we expect to exacerbate *SNCA*-driven pathology and related phenotypes; characterization of aged cohorts is in process. *LRRK2* knock-in models express the T1348N, D1994A, or A2016T mutations. Transgenic models over-express human *LRRK2* with the R1441G, R1441C, G2019S, Y1699C mutations. The repository also makes available mouse strains useful for research such as those expressing GFP, Cre, tTA and optogenetic proteins. Here, we specifically focus on the comparison of transgenic *SNCA* and *LRRK2* models in order to better inform the community as to which models are more appropriate for a specific purpose. Transgenic *SNCA* message and protein levels in striatum and cortex are being assayed in cohorts at 4, 8 and 12 months of age, and *LRRK2* levels are being assayed at a single age. All information will be made freely available via publication and the web. We continue to seek novel models of PD. For further information see https://www.jax.org/parkinsons.

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Poster

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Topic: C.03. Parkinson's Disease

Support: NIH Grant P50 AG047266

Society for Neuroscience, Neuroscience Scholars Program

Title: *In vivo* structural abnormalities and decreased free water in mice showing increased Aβ42 accumulation

Authors: *L. M. COLON-PEREZ, K. TORROELLA, E. OFORI, Y. LEVITES, P. CHAKRABARTY, D. VAILLANCOURT, T. GOLDE, M. FEBO;
Univ. of Florida, Gainesville, FL

Abstract: Abnormal β-amyloid (Aβ) accumulation is implicated in Alzheimer's disease (AD) pathogenesis and may partly underlie structural and diffusion abnormalities detected using MRI. However, a direct relationship between Aβ accumulation, particularly the toxic Aβ42 fragment, and imaging-based pathologies remains difficult to establish. Imaging mutant mouse strains bred to selectively overproduce toxic Aβ (Bri2- Aβ42 mouse) could offer important insight in this regard. In the present study, we assessed multiple structural features of Bri2- Aβ42 mice and age and sex matched wildtype (wt) controls. In order to assess *in vivo* interactions between Aβ42 overproduction and neuroinflammation, diffusion images were processed for free water (FW) signal. Diffusion images, fluid attenuation inversion recovery (FLAIR) and T2 weighted images were collected at 11.1 Tesla. The following scan parameters were used: (1) FLAIR image resolution: 0.10 x 0.10 x 0.75 mm^3, TR/TI/TE = 5000/1500/12 ms, (2) T2-weighted image resolution: 0.10 x 0.10 x 0.75 mm^3, TR/TE = 2000/32.5 ms, and (3) diffusion weighted images image resolution: 0.15 x 0.15 x 0.75 mm^3, TR/TE = 3000/25.9 ms, 30 directions with b = 1000 s/mm^2, and one b = 0. In our preliminary analyses, images were co-registered using FMRIB software library's linear registration tool (*flirt*). T2 images were used to estimate ventricular volumes, FLAIR scans were used to assess tissue hyperintensities, and diffusion scans were processed for FW, fractional anisotropy (FA) and mean diffusivity (MD). T2 scans showed a much larger ventricular size in Bri2-Aβ42 mice compared to wt. FLAIR images revealed hyperintensities surrounding and inside ventricles in Bri2-Aβ42 mice. Diffusion MRI showed a decrease in FW index in Bri2-Aβ42 (0.26 ± 0.04) compared to wt mice, as well as a larger FA values in Bri42-Aβ42. No differences were observed in MD. The reported effects were observed in whole brain aged (>14 month old) mice and at the moment we are carrying out atlas based ROI analysis and analyzing effects of age (<8 month) and sex. Our present preliminary results provide evidence that Aβ42 production results in structural abnormalities such as enlarged

ventricles with surrounding hyperintensities. In addition, a potential link to neuroinflammation is supported by greater FW in Bri2-Aβ42 than in wt mice. In addition to translating results in animal models of toxic Aβ42 to understand mechanisms underlying MRI findings in AD subjects, these biomarkers are expected to offer a powerful approach for determine *in vivo* therapeutic efficacy.

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Poster

698. Modeling Parkinson's Disease

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Topic: C.03. Parkinson's Disease

Title: Gut-Brain Axis: Long-distance propagation and seeding of the Lewy pathology in an *In vivo* model of Parkinson's disease

Authors: *M. DIEPENBROEK[1], J. M. PORTELA DOMINGUES[1], L. BOUSSET[2], R. MELKI[2], J.-Y. LI[1];
[1]Dept. of Exptl. Med. Sci., Wallenberg Neurosci. Ctr., Lund, Sweden; [2]Lab. d'Enzymologie et Biochimie Structurales, Ctr. Natl. de la Recherche Scientifique, Gif–sur–Yvette, France

Abstract: Clinical diagnosis of Parkinson's disease (PD) correlates to the topographic extent and severity of the Lewy pathology. Lewy pathology first appears in the peripheral nervous system (PNS) and the lower regions of the central nervous system (CNS), long before the typical motor symptoms of PD are evident. We previously reported that alpha synuclein (a-syn) and its aggregated forms, the key protein component in the Lewy pathology, could rapidly be transported from the intestine to the dorsal motor nucleus of the vagus of the medulla oblongata in rats. However, it is not clear whether the propagated a-syn can induce a-syn aggregation of the recipient neurons via a seeding effect after long distance transport from the peripheral tissues. Here, we report the propagation and seeding effects of exogenous a-syn species over a long time period *in vivo* in a-syn-overexpression or normal conditions. We injected recombinant human a-syn fibrils or brain lysates of a PD patient into the small intestinal wall of 2 months old C57/Bl6 mice or Bacterial Artificial Chromosome (BAC) transgenic PD mouse model overexpressing human a-syn fused to green fluorescent protein (GFP) under control of the mouse a-syn promoter.
Our preliminary data indicate that different a-syn species injected into the intestinal wall were transported over a long distance up to the brain. We observed elevated level of a-syn

accumulation at different levels of the brain in the a-syn injected BAC-a-syn-GFP animals (16 weeks after injection). Very interestingly, open field behavioral tests reveal a significant reduction in spontaneous locomotor activity after amphetamine administration in 10 months old C57/Bl6 as well as BAC-a-Syn-GFP mice (28 weeks after the injection) compared to the mice that received PBS injection. More detailed analyses on the changes in morphology, protein chemistry and behaviors are ongoing.

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Poster

698. Modeling Parkinson's Disease

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Topic: C.03. Parkinson's Disease

Support: Department of Veterans Affairs VA-1101 BX001641

Title: Behavioral deficits in mice overexpressing human wild-type alpha synuclein: Exacerbation by elevated biogenic aldehydes

Authors: ***P. A. MARTINEZ**[1,3], V. E. MARTINEZ[1], E. FERNANDEZ[2,4], R. STRONG[2,4]; [1]Pharmacol., [2]Pharmacol. & Barshop Inst. for Longevity and Aging Studies, Univ. of Texas Hlth. Sci. Ctr. at San Antonio, San Antonio, TX; [3]Geriatric Research, Education, and Clin. Ctr. and Res. Service, [4]Geriatric Research, Educ. and Clin. Ctr. and Res. Service, South Texas Veterans Hlth. Care Syst., San Antonio, TX

Abstract: Parkinson's disease (PD) is a chronic and progressive neurodegenerative disease affecting ~1% of people over the age of 65 years. A hallmark feature of PD is the presence of Lewy bodies (LBs) in surviving nigrostriatal dopamine neurons that are primarily composed of the presynaptic protein, alpha-synuclein (αSyn). While the exact cause of PD is unclear, studies indicate that a combination of genetic and environmental factors contribute to the neuropathology of PD. αSyn which is recognized as having a main role in neurotransmitter release, was first implicated in PD when point mutations and multiplication of the αSyn (SNCA) gene were linked to familial PD. Despite these findings, 90% of PD cases are idiopathic which have led to investigations of human wildtype αSyn transgenic mouse models of PD. Similar to mutant αSyn, wildtype αSyn localizes in the LB perimeter. The overexpression of αSyn leads to neurotoxicity in several cell lines and motor deficits resembling a parkinsonian phenotype that are responsive to L-dopa treatment in animals. The neurotoxicity of αSyn is believed to arise

through the formation of toxic oligomers. Under conditions of oxidative stress, natively folded αSyn can be driven to oligomer formation in the presence of toxic aldehydes. The dopamine metabolite, 3,4-dihydroxyphenylacetaldehyde, and the lipid peroxidation end product, 4-hydroxynonenal, are elevated in the PD brain and reported to promote aSyn oligomerization. In addition, reduced expression or polymorphisms in genes coding for the two enzymes primarily responsible for the detoxification of aldehydes in dopamine neurons, aldehyde dehydrogenase (ALDH)1a1 and ALDH2, are also implicated in PD. We previously reported that Aldh1a1$^{-/-}$x Aldh2$^{-/-}$ knockout mice exhibit neuropathological manifestations of PD, including elevated biogenic aldehydes, motor deficits that are ameliorated by L-dopa, and the loss of TH-immunoreactive neurons. The current study tests the hypothesis that αSyn is mechanistically related to the manifestations of PD that result from elevated biogenic aldehydes. To test this hypothesis, we generated mice that are null for both Aldh1a1 and Aldh2 and that overexpress human wildtype αSyn. Motor function was measured by examining performance on the accelerated rotarod and by gait analysis, and measures of grip strength. The results show that mice with elevated biogenic aldehydes, in the presence of overexpressed human wild-type aSyn, have more severe motor deficits as compared to mice that only overexpress aSyn. These data are consistent with the idea that elevated biogenic aldehydes impair motor function through mechanisms involving aSyn.

Disclosures: P.A. Martinez: None. **V.E. Martinez:** None. **E. Fernandez:** None. **R. Strong:** None.

Poster

699. Rodent Models of Parkinson's Disease and Related Disorders

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 699.01/V5

Topic: C.03. Parkinson’s Disease

Support: Michael J Fox Foundation

USF College of Pharmacy

Title: Arginase overexpression thwarts synuclein pathology in animal models of lewy body dementia

Authors: J. HUNT[1], L. SANDUSKY[1], W. FRASER[1], M.-L. SELENICA[1], J. BAKER[2], C. DICKEY[2], K. NASH[3], *D. C. LEE[1];
[1]Col. of Pharm. & Pharmaceut. Sci., [2]Col. of Med. Mol. Med., [3]Col. of Med. Mol. Pharmacol. & Physiol., USF Byrd Alzheimer's Inst., Tampa, FL

Abstract: Synucleinopathies comprises of neurodegenerative disease the harbor alpha synuclein pathology and associated with Parkinson's disease, Lewy body dementia, and multiple system atrophy. Pathways the govern inflammation and the by products generated play a pivotal role in disease outcomes. We find that arginase 1 overexpression using adeno associated virus (AAV9) in models of tauopathies reduce many aspects of the tau phenotype. Herein, we overexpressed arginase 1 (Arg1) via AAV9 in the CNS of alpha synuclein (α-syn) transgenic mice harboring the human α-syn A53T mutant (tetO-SNCAA53T**)** under the control of a tetracycline (tet) responsive element and driven by Camk2a. Mice express α-syn in the forebrain and hippocampus mimicking a lewy body dementia model. At 4 months α-syn mice and non-transgenic littermates received an injection of AAV9-Arg1 or AAV9-GFP in the hippocampus and anterior cortex. Mice were allowed to survive for duration of 8 months and received battery of cognitive test and affective processing test. We found significant behavioral deficits in α-syn mice compared to non-transgenic littermates consisting of spatial working memory, fear associated memory, affective processing, anxiety measures, and compulsion. Arg1 expression reversed some but not all the behavioral deficits in α-syn mice compared to GFP controls. Additionally, Arg1 significantly reduced inflammation and several forms of α-syn including total α-syn and phospho S129, and Tyr136. Several synaptic markers increased in α-syn mice treated with Arg1 compared to AAV9-GFP. Arg1 reduced total arginine content while increasing orthinine in Arg1 treated mice in both genotypes. All together, these data reveal a potential therapeutic pathway associated with arginine metabolism and synucleinopathies. Additionally, arginine metabolism may serve as a novel target to treat a wide array of neurodegenerative disorders.

Disclosures: J. Hunt: None. **L. Sandusky:** None. **W. Fraser:** None. **M. Selenica:** None. **J. Baker:** None. **C. Dickey:** None. **K. Nash:** None. **D.C. Lee:** None.

Poster

699. Rodent Models of Parkinson's Disease and Related Disorders

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 699.02/V6

Topic: C.03. Parkinson's Disease

Title: Cognitive Impairment in Glucocerebrosidase (GBA) deficient mouse models

Authors: *A. HAM;
Dept. of Neurol., Columbia Univ., New York, NY

Abstract: Glucocerebrosidase (GBA) is a lysosome hydrolase encoded by *GBA1* gene, a newly identified susceptibility gene associated with Parkinson's disease (PD) and Lewy body

disease(LBD), with GBA protein identified as one important component of Lewy bodies. Homozygous mutations in *GBA1*cause Gaucher disease, the most prevalent lysosome storage disease, and heterozygous mutations in GBA1 constitute the most frequent risk factors for PD. Emerging evidence has linked GBA mutations to cognitive dysfunction in PD, but the precise mechanisms whereby GBA mutations mediate cognitive dysfunction remain unclear. This study is designed to explore the role of GBA1 gene mutations in hippocampal associated learning and memory using GBA1 L444P heterozygous mutant mice as well as CamKCre mediated neuronal specific GBA1 conditional knockout mouse model. We have found that both GBA1 mutant and GBA1 conditional KO mice show significant deficits in working memory in a Morris Water Maze test and in feared conditions. Our study indicates that GBA1 gene deficiency may contribute to cognitive impairment in PD patients.

Disclosures: A. Ham: None.

Poster

699. Rodent Models of Parkinson's Disease and Related Disorders

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

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Topic: C.03. Parkinson’s Disease

Support: Parkinson's Disease Foundation

American Academy of Neurology

DSF Charitable Foundation

Blechman Family Foundation

Title: A novel, progressive, endogenous synucleinopathy model of Parkinson disease in rats

Authors: *A. D. VAN LAAR[1,2], K. R. WEBB[3], E. A. BURTON[2,1], J. T. GREENAMYRE[2,1]; [1]Neurol., Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA; [2]Pittsburgh Inst. for Neurodegenerative Dis., [3]Neurosci., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: One of the greatest obstacles in developing effective neuroprotective therapeutics for Parkinson disease (PD) is lack of a predictive preclinical research model that replicates the human disease with fidelity. We now report a new rat model in which brief pesticide exposure causes progressive accumulation and aggregation of endogenous α-synuclein, culminating in a delayed and progressive behavioral and pathological parkinsonian phenotype over a period of months. Lewis rats (6-9 months old) received baseline behavioral testing and then were treated

with rotenone (i.p.) once daily for 5 days only. During treatment, rats became mildly parkinsonian, but there was no morbidity or mortality. All rats recovered to their behavioral baseline over the succeeding week. They remained behaviorally normal until about 3 months, at which point all rats began to show mild progressive parkinsonian symptoms, including postural instability and bradykinesia. From onset, symptoms progressed over 3-4 months and stabilized thereafter. Pathological studies indicate that during the quiescent latent period before symptom onset, nigrostriatal neurons accumulate α-synuclein, which becomes progressively consolidated into inclusions by 3 months. The accumulation of α-synuclein is accompanied by progressive microglial activation - and many microglia also contain intracellular α-synuclein, apparently derived from nigral neurons. By the time of symptom onset, there is loss of nigrostriatal dopamine neurons, which continues to progress over a period of months. By 9 months, there is α-synuclein accumulation in other brain regions, including in the cortex, and there are legitimate Lewy bodies in some remaining nigral neurons. These results indicate that a remote environmental exposure has the potential to set in motion a pathological cascade that results, after a long latent period in parkinsonism. The model has many advantages over conventional models, including the fact that (i) it is a spontaneously progressive endogenous synucleinopathy, and (ii) potential disease-modifying treatments can be started at symptom onset, which is analogous to current clinical practice.

Disclosures: A.D. Van Laar: None. **K.R. Webb:** None. **E.A. Burton:** None. **J.T. Greenamyre:** None.

Poster

699. Rodent Models of Parkinson's Disease and Related Disorders

Location: Halls B-H

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Program#/Poster#: 699.04/V8

Topic: C.03. Parkinson’s Disease

Support: Canadian Institute of Health Research

Natural Research and Engineering Research Council of Canada

Title: Modulation of central immune responses in aged LRRK2 G2019S overexpressing mice following sub-chronic administration of paraquat

Authors: *Z. DWYER, C. RUDYK, S. HAYLEY;
Neurosci., Carleton Univ., Ottawa, ON, Canada

Abstract: Parkinson's disease (PD) is the second most common neurodegenerative disease of which the primary risk factor is age, wherein the majority of clinical diagnoses occur between the ages of 55 and 65. Recent research has focused on the role of microglia in the generation and propagation of pro-inflammatory responses which are believed to be an important factor in PD related dopaminergic cell death. The prevailing hypothesis postulates that cumulative insults to the brain and immune system over many years on a background of genetic susceptibility results in dopaminergic degeneration. One such genetic vulnerability is the Leucine Rich Repeat Kinase (LRRK2) G2019S mutation. LRRK2 is the most commonly mutated PD related gene and is implicated in nearly 10% of familial and 1% of sporadic PD cases and recent evidence strongly suggests that LRRK2 is highly expressed in microglia. While several genetic and environmental insults have been tested in conjunction with LRRK2 relatively little *in vivo* work has been done. One previously untested environmental factor thought to contribute to the development of PD is paraquat, a herbicide which when metabolized produces intracellular oxidative stress, thereby contributing to the chronic neuroinflammation found in PD patients. In this current study we sought to determine whether the LRRK2 G2019S mutation interacted with age or a sub-chronic paraquat injection regime to produce Parkinsonian symptoms and neuropathology. Aged animals demonstrated a variable reaction to the paraquat including significant motor and weight decreases, however, the G2019S mutation did not appear to produce or enhance any behavioral deficits. Analyzing neuropathological hallmarks of PD we found that paraquat induced age-related alterations in inflammatory states but that the G2019S mutation did not interact with these findings. Overall while age and paraquat seem to induce neuroinflammatory reactions and dopaminergic cell loss, LRRK2 G2019S overexpression does not appear to modulate these effects.

Disclosures: Z. Dwyer: None. **C. Rudyk:** None. **S. Hayley:** None.

Poster

699. Rodent Models of Parkinson's Disease and Related Disorders

Location: Halls B-H

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Program#/Poster#: 699.05/V9

Topic: C.03. Parkinson's Disease

Title: Propagation and aggregation of alpha synuclein in murine models

Authors: K. R. WALKER, W. ARIAS, J. BERGER, N. CHIRICHELLA, B. S. NUNEZ, R. SPRINGER, K. CIRILLO, J. SANCHEZ-PADILA, G. TOMBAUGH, A. GHAVAMI, *S. RAMBOZ;
Psychogenics Inc., Tarrytown, NY

Abstract: Aggregation of mis-folded proteins is a feature common to many neurodegenerative diseases. Parkinson's disease is a progressive movement disorder which is characterized neuropathologically by the presence of intraneuronal Lewy bodies and Lewy neurites. Alpha synuclein is a highly soluble pre-synaptic protein that is implicated in vesicular trafficking. Mis-folded and aggregated α synuclein (fibrillar α synuclein) is a major component of both Lewy bodies and Lewy neurites. Experimental studies have demonstrated that cerebral injection of brain lysates from α synuclein aggegrate bearing transgenic mice into pre-symptomatic young transgenic mice accelerates formation of α synuclein pathology (Luk, K.C. et al., 2012). A growing body of evidence has emerged demonstrating that synthetic α synuclein fibrils (both human and murine) are capable of 'seeding' and propagating α synuclein pathology not only in α synuclein transgenic mouse models but importantly in non-transgenic (WT) neuronal cultures and mice (Luk, K.C. et al., 2012a ; Luk, K.C. et al., 2012b; Volpicelli-Daley, L.A. et al., 2014). PsychoGenics has extensive experience working with both human and murine synthetic alpha synuclein fibrils in a variety of murine neurodegenerative disease models. Stereotaxic administration of 5μg of murine pre-formed fibrils (Proteos Inc.) is sufficient to induce a progressive α synuclein pathology, reduced dopamine levels and motor deficits in murine models (Martinez, T. et al., SFN 2014). As a continuation of our previous study sponsored by the Michael J Fox Foundation, PsychoGenics has expanded the study in WT mice as well as other murine models to assess the behavioral and pathological consequences of α synuclein propagation and aggregation.

Disclosures: **K.R. Walker:** A. Employment/Salary (full or part-time): Psychogenics. **W. Arias:** A. Employment/Salary (full or part-time): Psychogenics. **J. Berger:** A. Employment/Salary (full or part-time): Psychogenics. **N. Chirichella:** A. Employment/Salary (full or part-time): Psychogenics. **B.S. Nunez:** A. Employment/Salary (full or part-time): Psychogenics. **R. Springer:** A. Employment/Salary (full or part-time): Psychogenics. **K. Cirillo:** A. Employment/Salary (full or part-time): Psychogenics. **J. Sanchez-Padila:** A. Employment/Salary (full or part-time): Psychogenics. **G. Tombaugh:** A. Employment/Salary (full or part-time): Psychogenics. **A. Ghavami:** A. Employment/Salary (full or part-time): Psychogenics. **S. Ramboz:** 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; PsychoGenics.

Poster

699. Rodent Models of Parkinson's Disease and Related Disorders

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 699.06/V10

Topic: C.03. Parkinson's Disease

Title: N acylphosphatydilethanolamines (napes): early players in parkinson's disease

Authors: *F. PALESE[1], S. PONTIS[1], A. BASIT[1], N. REALINI[1], A. ARMIROTTI[1], D. PIOMELLI[1,2];
[1]D3, Fondazione Inst. Italiano Di Tecnologia, Genova, Italy; [2]Departments of Anat. and Neurobio., Univ. of California Irvine, Irvine, CA

Abstract: N-acyl-phosphatidylethanolamines (NAPEs) are membrane phospholipids present in the brain and other tissues. They are cleaved by a selective phospholipase D (NAPE-PLD) to form bioactive fatty acyl ethanolamides (FAEs) such as the endocannabinoid anandamide (Ueda, 2010). NAPEs have been primarily studied for their role as FAE precursors, but their multiple effects are suggestive of independent signaling functions. For example, NAPEs organize amphitropic cell division proteins at specific sites on the membrane surface, and help terminate inflammation by reducing phagocytosis (Coulon, 2011). It is known that NAPE levels increase following brain injury (Moesgaard, 2000).
Parkinson's Disease (PD) is a chronic neurodegenerative disorder characterized by emotional, cognitive and motor symptoms. Its primary neural substrate is the progressive loss of dopaminergic neurons in the substantia nigra pars compacta, accompanied by intraneuronal inclusions termed Lewy bodies. The etiology of PD is unclear. Nevertheless, there is evidence that chronic neuroinflammation plays a central role.
To investigate the possible contribution of NAPEs to PD we injected the neurotoxin 6-OHDA (6-hydroxy-dopamine) in the caudate putamen of C57BL6/J mice. Control animals were given only vehicle (ascorbic acid-saline). 48 h after 6-OHDA injection the mice were killed and brain tissue was processed for lipid analysis. A significant upregulation of NAPEs was observed in the striatum of 6-OHDA mice, compared to controls.
We also examined the effects of 6-OHDA on NAPE production in the human neuroblastoma SH-SY5Y cell line. Cells were treated with 6-OHDA (100 μM) and harvested at different time points after treatment. NAPE levels were increased 6h after treatment. Consistent with this effect, qPCR and protein analyses revealed a reduction in NAPE-PLD mRNA and protein starting from 2h and 4h after treatment, respectively.
The results suggest that NAPE accumulation may be increased during the early stages of PD. Further studies are needed to fully test this hypothesis and determine the function of endogenously produced NAPEs in the pathogenesis of PD and other forms of neuroinflammation.

Disclosures: **F. Palese:** None. **S. Pontis:** None. **A. Basit:** None. **N. Realini:** None. **A. Armirotti:** None. **D. Piomelli:** None.

Poster

699. Rodent Models of Parkinson's Disease and Related Disorders

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Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 699.07/V11

Topic: C.03. Parkinson's Disease

Support: MJFF Grant 11014

Title: Stronger cortical spindles and less power variability in hippocampal ripples in a LRRK2 mouse model of Parkinson's disease

Authors: *J.-P. WIEGAND[1,2], K. GIES[3], M. BARTLETT[4,5], T. FALK[4,5], S. COWEN[1,3];
[1]Evelyn F. McKnight Brain Inst., [2]Dept. of Neurosci., [3]Dept. of Psychology, [4]Dept. of Neurol., [5]Dept. of Pharmacol., Univ. of Arizona, Tucson, AZ

Abstract: The LRRK2 mutation is the most common genetic cause of Parkinson's disease (PD). Despite this, no study to date has investigated its impact on network-level neural activity. Recent data from Beccano-Kelly et al. (2014) suggest that LRRK2 knock-in mice exhibit an increase in glutamatergic release in cortical neurons. Such changes could significantly alter cortico-thalamic networks and enhance oscillatory activity produced by cortico-thalamic interactions.
Objective: We hypothesized that sleep-spindle oscillations are enhanced in LRRK2 knock-in mice. To investigate this question, we compared ripple and spindle activity recorded from LRRK2 knock-in and wild-type mice. **Methods:** 5 LRRK2 G2019S and 9 WT C57bl/6 male mice (The Jackson Laboratory) were implanted with depth electrodes and surface EEG electrodes. Depth electrodes were implanted into the motor cortex (M1), anterior cingulate cortex (ACC), hippocampus and striatum. Surface electrodes were implanted above somatosensory (S1) and visual cortex (V1). We recorded neural activity during sleep periods preceding and following an open-field foraging task and novel object exposure. Activity was recorded at 20kHz and down-sampled to 2kHz. We filtered for spindle activity (9-16Hz) that exceeded 2.5 std above the mean power and ripple activity (80-180Hz) that exceeded 5.5 std above the mean power.
Results: LRRK2 mice expressed a significant increase in the power of both early and late peak spindle frequency (dB/Hz) relative to controls ($p < 0.05$, Student's t-test) in all cortical regions (M1, ACC, S1, and V1). In contrast, no difference in the distributions of peak spindle frequencies and durations was observed between LRRK2 and wild-type mice. Moreover, preliminary results from our analysis indicate that ripple-to-ripple variance in the power of each ripple event is reduced in LRRK2 relative to WT mice ($p < 0.05$, Student's t-test). **Conclusions:** Our results support the conclusion that the LRRK2 G2019S mutation results in lasting alterations in two sleep-associated patterns of neural activity that are linked to memory consolidation. Because cortical sleep spindles and ripple oscillations are highly preserved across species, these alterations could serve as a diagnostic biomarker for LRRK2 PD.

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Poster

699. Rodent Models of Parkinson's Disease and Related Disorders

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 699.08/V12

Topic: C.03. Parkinson's Disease

Title: Micronized palmitoylethanolammide: a new pharmacological strategy to prevent neurodegenerative diseases associated to the old age

Authors: ***R. CRUPI**[1], M. CAMPOLO[2], R. SIRACUSA[3], D. IMPELLIZZERI[3], M. CORDARO[3], G. CASILI[3], E. ESPOSITO[4], S. CUZZOCREA[4];
[1]Biol. and Envrn. Sci., [2]Univ. of Messina, Messina, Italy; [3]Uniersity of Messina, Messina, Italy; [4]Unirersity of Messina, Messina, Italy

Abstract: Parkinson's disease (PD) is a disorder resulted by degeneration of dopaminergic neurons. The common symptoms of the disease are: resting tremor, rigidity, and hypokinesia, with onset asymmetrical. At the moment there is no cure. Ageing remains the biggest risk factor for developing idiopathic PD. Recent studies have already demonstrated the neuroprotective effects of Palmitoylethanolamide (PEA), alone or in combination with antioxidants, in an experimental model of PD after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induction.
The aim of the present study was to evaluate the pretreatment effect of micronized PEA formulation (PEA-m), on the neuroinflammation process and on the neuronal death on *in vivo* model of PD on aged mice. The old animals were pretreated for 60 days with PEA-m at dose of 10mg/kg. After pretreatment, they received four injections of the dopaminergic neurotoxin MPTP and were sacrificed 7 days after induction. On the 8th days, brains were processed for histological and immunohistochemical analysis. Pretreatment with PEA-m significantly ameliorated behavioral deficits, reduced the expression of specific markers of PD such as tyrosine hydroxylase (TH), dopamine transporter (DAT), as well as decreased the upregulation of α -synuclein and β-tubulin in the substantia nigra after MPTP induction. In addition, the strong increase in neuroinflammation, detected by GFAP, Iba-1, TNF-α, IL-1β expression and the neurotrophic factors levels such as BDNF and NGF were evaluated.
In conclusion, we demonstrated that the pretreatment with PEA-m in aged mice was able to ameliorate the development of PD disease. Thus, this strategy could prevent neurodegenerative diseases associated to the old age.

Disclosures: **R. Crupi:** None. **M. Campolo:** None. **R. Siracusa:** None. **D. Impellizzeri:** None. **M. Cordaro:** None. **G. Casili:** None. **E. Esposito:** None. **S. Cuzzocrea:** None.

Poster

699. Rodent Models of Parkinson's Disease and Related Disorders

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Topic: C.03. Parkinson's Disease

Support: NIH-N5074730

Title: Induction of alpha-synuclein pathology by neonatal delivery of adeno-associated virus.

Authors: ***M. DELENCLOS**[1], A. KURTI[1], M. YUE[1], J. D. FRYER[1,2], P. J. MCLEAN[1,2]; [1]Mayo Clin., Jacksonville, FL; [2]Mayo Grad. School, Mayo Col. of Med., Jacksonville, FL

Abstract: Abnormal accumulation of alpha synuclein (α-syn) is a pathological hallmark of Lewy body related disorders such as Parkinson's disease (PD) and Dementia with Lewy body disease (DLB). Over the years myriad of animal models have been developed to mimic pathological features of synucleinopathies by over-expressing human α-syn under a variety of promoters. Although different strategies have been used, most models have little or no reliable and predictive phenotype. Novel animal models are a valuable tool for understanding neuronal pathology and to facilitate development of new therapeutics for these diseases. Here, we report the development and characterization of a novel model in which mice express wild-type α-syn via somatic brain transgenesis mediated by adeno-associated virus (AAV). After 1, 3, and 6 months of age following intracerebroventricular (ICV) injection, mice were subjected to a battery of behavioral tests followed by pathological analyses of the brains. Remarkably, significant levels of αsyn expression are detected throughout the brain as early as 1 month old including olfactory bulb, hippocampus, striatum, and cerebellum. Immunostaining with a phospho-αsyn (p-αsyn) specific antibody reveals abundant p-αsyn expression and pathologic α-syn is detected using the disease specific antibody 5G4. However, no abnormal behavior or motor dysfunction could be observed in the 3 months of age group when performing rotarod, beam walk and pole test. Longer cohorts and behavioral tests for motor performance and memory tasks are still ongoing. We predict that whole brain transduction of neonatal mouse will recapitulate pathological, neurochemical, and behavioral features of Lewy body disorders by 6 months.

Disclosures: **M. Delenclos:** None. **A. Kurti:** None. **M. Yue:** None. **J.D. Fryer:** None. **P.J. McLean:** None.

Poster

699. Rodent Models of Parkinson's Disease and Related Disorders

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the American Parkinson Disease Association (APDA) Center for Advanced PD Research at Washington University

the Greater St. Louis Chapter of the APDA

the McDonnell Center for Higher Brain Function

Title: Progressive loss of dopamine function in a primate model of Parkinson disease

Authors: *J. S. PERLMUTTER[1], L. TIAN[2], S. K. LOFTIN[2], H. P. FLORES[2], S. A. NORRIS[2]; [1]Washington Univ. Sch. Med., Saint Louis, MO; [2]Washington Univ. Sch. of Med., St. Louis, MO

Abstract: Objective: Parkinson disease (PD) is a progressive neurological disease involving loss of nigrostriatal neurons in the substantia nigra (SN). Unilateral internal carotid delivery of a single dose of MPTP produces nigrostriatal damage but the time-course of this injury remains to be determined. This study was designed to measure the time-dependent change in the nigrostriatal dopamine system in this primate model of PD. **Methods:** Fifteen macaques had baseline MRI and PET to assess presynaptic dopaminergic nigrostriatal neurons: 6-[^{18}F]fluorodopa (FD; primarily reflects decarboxylase activity), [^{11}C]dihydrotetrabenazine (DTBZ; reflects vesicular monoamine transporter type 2 [VMAT2]), and 2beta-[^{11}C]carbomethoxy-3beta-(4-fluorophenyl)tropane (CFT; reflects membranous dopamine transporter [DAT]), then received a single dose of unilateral intracarotid 0.25mg/kg of MPTP. PETs were repeated after 10, 21, 30, 60 days. Animals were trained to perform several behavioral tasks to assess motor abilities, and ratings were done before and after MPTP. The animals were euthanized at different time points for measurements of striatal AADC enzymatic activities, striatal dopamine concentration and unbiased stereological counts of tyrosine hydroxylase (TH)-immunoreactive (ir) neurons in SN. The influx constant (K_{occ}) for FD and non-

displaceable binding potentials (BP_{ND}) for CFT and DTBZ were calculated for the caudate and putamen using an occipital reference region. **Results:** Motor parkinsonism increased at 10 days and reached maximum at 21 days then remained stable until euthanasia (Skillings-Mack test: p = 0.02, n = 18). The striatal PET measures decreased over time (Skillings-Mack test: p = 0.02, n = 16; p = 0.007, n = 16; p = 0.03, n = 16; for FD *K*occ , CFT BP_{ND} and DTBZ BP_{ND}, respectively). **Conclusions:** Taken together these results demonstrate a time-dependent change *in vivo* measures of striatal dopaminergic function from a single dose of MPTP. These results indicate the importance of considering these time-dependent changes in studies of biomarkers reflecting nigrostriatal function or interventions to alter nigrostriatal injury.

Disclosures: J.S. Perlmutter: None. **L. Tian:** None. **S.K. Loftin:** None. **H.P. Flores:** None. **S.A. Norris:** None.

Poster

699. Rodent Models of Parkinson's Disease and Related Disorders

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Program#/Poster#: 699.11/V15

Topic: C.03. Parkinson's Disease

Title: N-acylethanolamine acid amidase (NAAA) inhibitors are protective in a model of Parkinson's disease.

Authors: *N. REALINI[1], S. PONTIS[1], F. PALESE[1], M. MIGLIORE[1], A. ARMIROTTI[1], E. ROMEO[1], M. SUMMA[1], R. SCARPELLI[1], D. PIOMELLI[2,1];
[1]Italian Inst. of Technol., Genova, Italy; [2]Departments of Anat. and Neurobiology, Pharmacol. and Biol. Chem., Univ. of California, Irvine, CA

Abstract: NAAA is lysosomal cysteine hydrolase that catalyses the biodegradation of palmitoylethanolamide (PEA) and oleoylethanolamide (OEA) (Tsuboi et al., 2005; Ueda et al., 2010), two endogenous lipid amides that suppress inflammation by activating the ligand-operated transcription factor, peroxisome proliferator-activated receptor-α (PPAR-α) (Pontis et al., 2016). Resident macrophages and other host-defense cells constitutively generate PEA and OEA in amounts that are sufficient to fully engage PPAR-α (Piomelli and Sasso, 2014). This process is halted during inflammation, however, leading to a decrease in PPAR-α-mediated signaling and an acceleration of the inflammatory response (Bandiera et al., 2014). Accordingly, small-molecule NAAA inhibitors restore normal PEA and OEA levels in inflamed tissues and exert profound anti-inflammatory effects in animal models, pointing to NAAA as a potential target for therapy (Bandiera et al., 2014). Even though NAAA may offer a new target for anti-inflammatory therapy, the lipid-like structures and reactive warheads of current NAAA inhibitors

limit the use of these agents as oral drugs. Here, we describe a series of novel benzothiazole-piperazine derivatives that inhibit NAAA in a potent and selective manner via a non-covalent mechanism. In vitro and in vivo experiments indicate that a representative member of this class, ARN19702, is potent, selective for NAAA, and orally available. ARN19702 crosses the blood-brain barrier and elevates PEA and OEA levels in the CNS. Based on the promising in vivo profile of this compound, we tested the effect of ARN19702 treatment in the 6OHDA mouse model of Parkinson's disease (PD): a 21-day treatment with ARN19702 (30 mg-kg-1, twice daily, i.p.) elicited a a marked protective effects, preventing the behavioral and neurodegenerative effects of 6-OHDA injections. In conclusion, the compound ARN19702 exemplifies a second generation of non-covalent NAAA inhibitors that may be useful in the treatment of PD and other chronic CNS disorders.

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Poster

699. Rodent Models of Parkinson's Disease and Related Disorders

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 699.12/V16

Topic: C.03. Parkinson's Disease

Support: Brown University UTRA Fellowship

NIGMS R01GM067862

DGE 0966060

Title: Manipulating mitochondrial decay and regeneration in a *Drosophila* model of neurodegeneration

Authors: ***D. YOON**[1], A. N. SPIERER[2], D. M. RAND[2];
[1]Dept. of Neurosci., [2]Dept. of Ecology and Evolutionary Biol., Brown Univ., Providence, RI

Abstract: Nearly one in six individuals will develop a neurodegenerative disorder in their lifetime, according to the WHO. Olfactory dysfunction may serve as an early indicator of neural decline and progression in several disorders, including Alzheimer's and Parkinson's disease. Additionally, there is evidence that mitochondrial dysfunction plays a role in the progression of such neurological disorders. We aimed to model neurodegeneration in *Drosophila* using the Gal4-UAS system to express a mitochondrially-targeted restriction enzyme to cleave mtDNA in olfactory receptor neurons. To confirm the efficacy of this model we first expressed the mitochondrially-targeted restriction enzyme in the progenitor germline cells, alone, and in combination with *Spargel,* a gene that upregulates mitochondrial biogenesis. Flies overexpressing the restriction enzyme against mtDNA in their germlines had extremely low fertility compared to those of the various control crosses. Flies expressing both the mitochondrial restriction enzyme and *Spargel* constructs showed intermediate levels of fertility relative to the controls. This suggests that increased mitochondria biogenesis can rescue the cellular and tissue defects associated with linearized mtDNA. We sought to apply this system in an olfactory paradigm by screening for differences in chemotactic response to apple cider vinegar and ethanol. Using four olfactory Gal4 drivers, we identified a target Gal4 line for which expression of the mtDNA restriction enzyme significantly altered the preference of flies for ethanol, in both males and females, when compared to a expression of a GFP control. These findings serve as the foundation for future work dissecting the role of mitochondria in neurological health and decline via manipulation of mitochondrial turnover dynamics that may enhance existing neurodegenerative treatments.

Disclosures: D. Yoon: None. **A.N. Spierer:** None. **D.M. Rand:** None.

Poster

699. Rodent Models of Parkinson's Disease and Related Disorders

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Program#/Poster#: 699.13/V17

Topic: C.03. Parkinson's Disease

Title: Towards rodent and nonhuman primate models of multiple system atrophy

Authors: *D. J. MARMION[1], R. J. MANDEL[2], D. KIRIK[3], Y. CHU[1], T. MCCOWN[4], S. J. GRAY[5,4], J. H. KORDOWER[1,6];
[1]Dept. of Neurolog. Sci., Rush Univ. Med. Ctr., Chicago, IL; [2]Dept. of Neurosci., Univ. of Florida, Gainesville, FL; [3]Dept. of Exptl. Med. Sci., Lund Univ., Lund, Sweden; [4]Gene Therapy Ctr., [5]Dept. of Ophthalmology, Univ. of North Carolina, Chapel Hill, NC; [6]The Van Andel Inst., Grand Rapids, MI

Abstract: Multiple system atrophy (MSA) is a synucleinopathy in which alpha synuclein preferentially aggregates in oligodendroglia. To date, only murine models of MSA exist for the study of this disease. Towards this end, we sought to develop novel rat and nonhuman primate models of MSA by overexpressing alpha synuclein in oligodendroglia cells using a novel oligotrophic adeno-associated virus (AAV). vector where expression was driven by a CBA promoter. Initially, rats received unilateral injections of this novel AAV–CBA-GFP vector (AAV-Oligo-GFP) stereotaxically in the striatum. After 3-months, rodents were sacrificed and histological methods were used to assess the specificity of the viral vector. Our data shows 30,000-35,000 GFP-positive cells in the striatum, with 94-97% of the GFP-positive cells co-localizing with oligodendroglial marker Olig2. There was little or no co-expression in NeuN (2.9-4.7%) or GFAP (0.18-0.49%)-positive cells. We next sought to test the efficacy of this vector in nonhuman primates. Three rhesus macaques received intrastriatal injections of AAV-Oligo-GFP (1x10^13 vg/mL) unilaterally (3 in the putamen and 2 injections in the caudate nucleus). The animals were sacrificed after 1 month and analyzed using histological and stereological methods. As in the rodents, stereological estimates of transfected profiles revealed a large number of striatal cells expressing GFP (monkey 1: 639,877; monkey 2: 630,520; monkey 3: 161,948) in the striatum. Additionally, 90-94% of the GFP expressing cells co-localized with Olig2, with sparse co-localization with either NeuN (0.23-1.42%) or GFAP (0.09-0.12%). Finally, we recently injected three monkeys into the striatum as before, with the vector expressing the alpha synuclein transgene (AAV-Oligo-α-syn). Histological analyses in all monkeys 3 months after injection demonstrated wide spread monomeric and aggregated alpha synuclein expression as determined by LB509 and serine 129 alpha synuclein immunoreactivity. This expression was preferentially seen in oligodendroglia. Quantitative analyses of the staining pattern in these monkeys is currently underway. These data support the establishment of a viral over expression model of MSA.

Disclosures: D.J. Marmion: None. **R.J. Mandel:** None. **D. Kirik:** None. **Y. Chu:** None. **T. McCown:** None. **S.J. Gray:** None. **J.H. Kordower:** None.

Poster

700. Parkinson's Disease: Biomarkers and Therapeutics

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 700.01/V18

Topic: C.03. Parkinson’s Disease

Title: The ninds parkinson's disease biomarkers program

Authors: *C. R. SWANSON[1], D. BABCOCK[1], K. DAVID[1], K. GWINN[1], B. LANDIN[2], J. LINDE[2], J. LIU[2], M. MCAULIFFE[2], B.-A. SIEBER[1], P. TIMOTHEE[2], C. ST. HILLAIRE-CLARKE[1], M. SUTHERLAND[1];
[1]NIH-NINDS, Bethesda, MD; [2]NIH-CIT, Bethesda, MD

Abstract: Parkinson's Disease (PD) is the second most-prevalent neurodegenerative disease, affecting more than 4 million individuals worldwide. Presently, there are no disease-modifying therapies available. The National Institute of Neurological Disorders and Stroke (NINDS) Parkinson's Disease Biomarkers Program (PDBP) is a consortium of scientists and movement disorder clinicians established to support biomarker discovery and advance biomarker utility in Phase II clinical trials for PD and related disorders. The PDBP collects standardized longitudinal clinical data as well as biospecimens from PD and Parkinsonism cases, and healthy controls. Currently, there are twelve hypothesis-driven projects supported within the PDBP, of which nine are clinical. As of April 2016, there are more than 1400 enrolled participants. Clinical sites follow standardized protocols for subject visits and biospecimen collection. The PDBP data management resource (DMR) catalogs data electronically, while allowing users to query for expansive clinical and biospecimen data. Moreover, through the DMR users can request biospecimens from the NINDS Repository at no cost, following review by the PD Biospecimen Resource Acquisition Committee (BRAC). Presently, there are more than 1300 DNA and 3500 longitudinal RNA samples available. Most of the DNA has been genotyped using the NeuroX chip and this data is available in the DMR. Furthermore, there are more than 2700 samples each of serum, plasma, and whole blood, as well as over 700 CSF samples collected over time which are available for analysis. Overall, the PDBP cohort serves as a well-standardized cohort of clinical information regarding how to request access to the DMR, query for data and samples, and order biospecimens can be found on the PDBP website (https://pdbp.ninds.nih.gov).

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Poster

700. Parkinson's Disease: Biomarkers and Therapeutics

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Topic: C.03. Parkinson's Disease

Support: Michael J. Fox Foundation

The Mayo Clinic

Title: DNA methylation and gene expression pattern analysis for Parkinson's disease blood biomarker discovery

Authors: *A. R. HENDERSON-SMITH[1], B. MEECHOOVET[1], A. L. SINIARD[1], E. DRIVER-DUNCKLEY[2], M. J. HUENTELMAN[1], T. L. DUNCKLEY[3];
[1]Translational Genomics Res. Inst., Phoenix, AZ; [2]Div. of Neurol., Mayo Clin., Scottsdale, AZ; [3]Ctr. for Neurodegeneration, Biodesign Institute, Arizona State Univ., Tempe, AZ

Abstract: Parkinson's disease (PD) is a progressive neurodegenerative disorder, diagnosed only at an advanced disease stage, by a series of motor deficits that manifest over years or decades. Aberrant epigenetic modifications, including hypomethylation of α-synuclein in PD, exist across a range of diseases, from cancer to schizophrenia, and are non-invasively detectable in many body fluids and blood tissue as markers of disease. We aimed to characterize DNA methylation and gene expression patterns in blood from PD patients and matched healthy controls to identify disease-specific biomarkers that may be used to aid earlier, more accurate disease diagnosis and tracking of disease progression.
Two whole-blood samples were collected from PD patients and healthy controls, one for DNA methylation detection and one for RNA sequencing. DNA methylation sites were probed with the Illumina Infinium HumanMethylation450 BeadChips and analyzed for differential methylation. We used the Illumina HiSeq2000 platform for RNA sequencing and performed differential expression analysis. Intersection analysis of DNA methylation and RNA-seq was done with Bedtools.
PD methylation profiles are readily distinguishable from healthy controls, even in whole blood DNA samples. Differential expression analyses of RNA-seq data identified global changes in gene regulation, including overall gene expression levels and expression levels of specific transcript splice variants. Combined methylation quantitative trait loci analyses (meQTL) identified cis-acting meQTLs associated with differential expression of proximal loci. Establishing clear patterns of altered disease-specific DNA methylation, RNA expression and processing, and meQTL analyses from whole blood, a non-invasive tissue collection option, provides increased promise for the development of a molecular biomarker for PD with sufficient sensitivity and specificity to aid in the diagnosis and tracking of this disorder.

Disclosures: A.R. Henderson-Smith: None. **B. Meechoovet:** None. **A.L. Siniard:** None. **E. Driver-Dunckley:** None. **M.J. Huentelman:** None. **T.L. Dunckley:** None.

Poster

700. Parkinson's Disease: Biomarkers and Therapeutics

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Topic: C.03. Parkinson's Disease

Support: NIH U24NS095871

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Some data and biospecimens were obtained from the Parkinson's Disease Biomarkers Program (PDBP) Consortium, part of the National Institute of Neurological Disorders and Stroke at the National Institutes of Health.

Title: Broad Range of biospecimens available from well characterized Parkinson Disease Subjects and Controls

Authors: *R. CASE[1], C. BALES[1], C. WEGEL[1], S. LASCH[2], K. MAREK[2], P. TIMOTHEE[3], D. BABCOCK[3], B.-A. SIEBER[3], M. SUTHERLAND[3], K. GWINN[3], A. REIMER[4], C. KOPIL[4], M. FRASIER[4], T. FOROUD[1];
[1]Indiana Univ., Indianapolis, IN; [2]Inst. for Neurodegenerative Disorders, New Haven, CT; [3]Natl. Inst. of Neurolog. Dis. and Stroke, Bethesda, MD; [4]Michael J. Fox Fndn., New York, NY

Abstract: The availability of uniformly collected biospecimens from extensively phenotyped subjects is essential for the identification of high quality biomarkers which can advance the development of therapeutic interventions for Parkinson disease (PD). The Michael J. Fox Foundation (MJFF) and the National Institutes of Neurological Diseases and Stroke (NINDS) are committed to obtain and distribute a range of biospecimens and associated clinical data collected under standard operating procedures from research subjects in the following groups: (1) PD patients at various stages of disease: early, moderate (mod), advanced (adv); (2) healthy controls (HC); and (3) other subgroups. These samples are banked within a single umbrella biorepository located at Indiana University. The biorepository at Indiana University houses biospecimens from multiple ongoing and closed studies. Some of the studies collect longitudinal (long) samples. The table below summarizes the studies and types of biospecimens banked at Indiana University. More information for particular studies can be obtained at www.michaeljfox.org/dataspecimens and https://pdbp.ninds.nih.gov/. Biospecimens collected under these protocols are now available and are being used in ongoing research studies. Researchers request biospecimens through an application process (https://pdbp.ninds.nih.gov/biospecimens) or http://www.ppmi-info.org/access-data-specimens/request-specimens/.

Study Summary

Study	Long	PD	Control	DNA	Plasma	Serum	RNA	CSF	Urine	Whole Blood
PPMI (MJFF)	X	Early: 400	200	X	X	X	X	X	X	X
BioFIND (MJFF/NINDS)		Mod/ Adv: 126	106	X	X	X	X	X	X	

DATATOP (MJFF)	X	Early: 800		X		X		X	X	
24Hour		Early: 24	11		X	X		X		X
PDBP (NINDS)	X	Early: 459 Mod/Adv: 486	510	X	X	X	X	X	X	X
* Recruitment is still ongoing for this study and/or subject group										

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Poster

700. Parkinson's Disease: Biomarkers and Therapeutics

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 700.04/W3

Topic: C.03. Parkinson’s Disease

Title: Using eye movements to identify early biomarkers of disease progression in Parkinson’s patients with and without LRRK2 gene mutations

Authors: *J. MORRIS[1], J. HUANG[1], D. BRIEN[1], B. COE[1], J. HUANG[2], N. VISANJI[2], T. GHATE[2], A. E. LANG[2], C. MARRAS[2], D. P. MUNOZ[1];
[1]Ctr. for Neurosci. Studies, Queen's Univ., Kingston, ON, Canada; [2]Morton and Gloria Shulman Movement Disorders Clin. and Edmond J Safra Program in Parkinson Dis., Univ. Hlth. Network, Toronto, ON, Canada

Abstract: In some patients with Parkinson’s disease (PD), variations of the Leucine-rich repeat kinase 2 (LRRK2) gene have been associated with the development of the disease. There is evidence suggesting the importance of identifying deficits in executive functioning in patients with PD, as it may lead to earlier diagnosis. One promising approach is to use eye tracking. Patients with PD exhibit specific deficits in voluntary saccade control, specifically the anti-saccade task, where participants must look in the opposite direction of a peripheral visual stimulus. This task assesses the ability to inhibit of the automatic response and requires a generation of a voluntary command to look in the opposite direction. Commonly interleaved with the anti-saccade task, the pro-saccade task assesses the basic sensory-motor processing of eye

movements via automatic tendencies to look at the peripheral visual stimuli. PD patients exhibit deficits include increased saccadic reaction times (SRTs) and direction errors during the anti-saccade task. There are also significant alterations of pupil responses during voluntary movement preparation in patients with PD. It is unclear whether LRRK2 mutation carriers before they manifest PD symptoms (LRRK2 carriers) exhibit the same behavioural and pupillary discrepancies as patients with PD. By exploiting these known behavioural variations, saccade tasks may be used as a diagnostic tool to help differentiate between the healthy population and pre-PD patients. We conducted interleaved pro- and anti-saccade trials with age-matched controls, patients with idiopathic PD, and LRRK2 carriers. Preliminary analysis showed no significant differences between the LRRK2 group and controls in SRTs and direction errors, although there were significant pupillary differences between the two groups. LRRK2 carriers had on average smaller baseline pupil size when starting the either saccadic task than healthy age-matched controls. The pupils of LRRK2 carriers also did not constrict as much as controls during both the pro- and anti-saccade task. This may be an indication that pupillary abnormalities may be a prodromal symptom of PD. Further analysis is essential to identify pre-symptomatic behavioural biomarkers of PD that accurately predict disease and thus lead to earlier PD detection.

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Poster

700. Parkinson's Disease: Biomarkers and Therapeutics

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Topic: C.03. Parkinson’s Disease

Support: Michael J. Fox Foundation

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R01DE022772

R21CA17553

Title: NLRP3 expression in distressed neurons and a NLRP3 polymorphism associated with decreased risk of Parkinson's disease

Authors: *K. VON HERRMANN[1], L. A. SALAS[1], E. M. MARTINEZ[1], W. W. FENG[1], W. F. HICKEY[2], B. C. CHRISTENSEN[1], S. L. LEE[1,2], M. S. FELDMAN[1,2], M. C. HAVRDA[1]; [1]Geisel Sch. of Med. At Dartmouth, Lebanon, NH; [2]Dartmouth-Hitchcock Med. Ctr., Lebanon, NH

Abstract: Neuroinflammation is a pathophysiology associated with Parkinson's disease (PD). Characterizing the cellular and molecular basis of neuroinflammation is critical to understanding the progression of PD. The NLRP3 inflammasome is a cell-intrinsic pro-inflammatory mediator capable of initiating inflammation. NLRP3, a key component of the multi-protein NLRP3 inflammasome complex, is a NOD-like receptor (NLR) containing a highly conserved NACHT domain along with an n-terminal pyrin domain and a c-terminal leucine rich repeat domain. NLRP3 activity has been associated with the progression of Alzheimer's disease, however the role of NLRP3 in PD is not known. Recent evidence that misfolded synuclein can activate NLRP3 *in vitro* suggests that the NLRP3 inflammasome might play an important role in the development and progression of PD. NLRP3 expression was examined in post-mortem CNS tissues from PD patients (n=17) and control subjects of similar age (n=11). Our histologic and biochemical findings indicate elevated NLRP3 expression in mesencephalic tissues from Parkinson's patients as compared with controls. NLRP3 immunoreactivity was readily detectable in pigmented neurons and Lewy neurites in PD patients indicating that degenerating neurons are a cell-of-origin for inflammasome activity in PD. We independently confirmed neuronal NLRP3 expression in distressed neurons proximal to areas of cerebral infarction and in differentiated SH-SY5Y cells treated with neurotoxins. Although PD most often occurs sporadically, increasing evidence points to underlying genetic predispositions that can alter the incidence and progression of PD. Having identified elevated levels of NLRP3 in DA neurons in the degenerating mesencephalon of PD patients, we undertook an evaluation of NLRP3 in the germline of PD patients and control individuals. In analyzing newly available exome sequencing data obtained from the Parkinson's Progression Markers Initiative (PPMI) database, we found multiple SNPs clustered in the NLRP3 NACHT domain with an individual SNP associated with a reduced risk of developing PD. We were able to use these data to differentiate Parkinson's patients from a population of SWEDD (scans without evidence of dopaminergic deficit) individuals. Our studies identify the NLRP3 inflammasome as a polymorphic neuron-intrinsic inflammatory mediator in Parkinson's and suggest that understanding NLRP3 polymorphisms may help distinguish PD from early stage disease or non-classical parkinsonism. In addition, these findings may provide the basis for novel therapeutic approaches aimed at modulating NLRP3 activity in neuroinflammatory disorders.

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Poster

700. Parkinson's Disease: Biomarkers and Therapeutics

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Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

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Topic: C.03. Parkinson's Disease

Support: NINDS U01

Title: H-ferritin in serum and cerebrospinal fluid in Parkinson's disease

Authors: *A. M. SNYDER[1], J. R. CONNOR[1], G. DU[2], C. STETTER[3], M. M. LEWIS[2], X. HUANG[2];
[1]Neurosurgery, MC H110, [2]Neurol., [3]Penn State Univ. Coll Med., Hershey, PA

Abstract: It is well established that the substantia nigra (SN) is an area of iron enrichment in the brain, with levels increasing with advancing age. In Parkinson's disease (PD), iron levels are further enhanced, yet the role that iron plays in disease progression remains enigmatic. Ferritin is a key iron sequestration protein and is comprised of H- and L- subunits. The ferroxidase activity of H-ferritin makes this protein of particular interest in PD because it may limit iron toxicity in a condition demonstrated to have iron overload. Data from autopsy tissue suggest that H-ferritin levels are decreased in PD, but H-ferritin levels in the central nervous system, as reflected by cerebrospinal fluid (CSF), or in the periphery are unknown in living PD patients. The overall goal of our work is to establish a biomarker profile to better understand the role of iron in PD pathophysiology. We hypothesize that the correlation between serum and CSF H-ferritin levels will be useful in understanding disease progression. We measured H-ferritin levels in serum and CSF from 109 control, 183 PD, and 12 PDism cases; the latter group includes multiple system atrophy and progressive supranucelar palsy. There was no relationship between serum and CSF H-ferritin levels in controls but PD subjects displayed a significant positive correlation. Sub-division of PD cases into disease duration categories of less than one year, one to five years, five to ten years, and over ten years, revealed that there was a significant positive correlation between serum and CSF in PD cases with disease duration of one to five years and greater than ten years, suggesting this correlation is dynamic rather than stable during disease progression. We also assessed H-ferritin immunostaining in the SN in autopsy sections from control, PD, and PDism cases. H-ferritin protein was found to strongly stain in glia in disease conditions but not in controls. These data suggest that iron management is not causative of PD pathology but does signify a shift in iron homeostasis two to five years after PD diagnosis and also in later stages of the disease. Investigation is ongoing to determine if H-ferritin levels have prognostic value for determining rate and severity of disease progression or if it is informative in PDism cases.

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Poster

700. Parkinson's Disease: Biomarkers and Therapeutics

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Topic: C.03. Parkinson's Disease

Support: Wenner-Gren Foundation

Title: Cerebrospinal fluid as a source of alpha-synuclein related biomarkers for detection of early stage Parkinson's disease

Authors: *W. P. PASLAWSKI, X. ZHANG, P. SVENNINGSSON;
Dept. of Clin. Neurosci., Karolinska Institutet, Stockholm, Sweden

Abstract: Parkinson's disease (PD) is affecting up to 10 million people worldwide and even though 200 years passed from the time when James Parkinson published "*An Essay on the Shaking Palsy"* we are still unable to cure the disease or detect it in the early stages. PD is characterized by progressive loss of dopaminergic neurons and presence of abnormal inclusion called Lewy bodies (LBs) in surviving neurons. Although several medication strategies exist against PD, they are aimed to slow down disorder progression or relieve the symptoms and none of them is able to stop or reverse the damage caused by the disease. More importantly, only few indirect methods are currently available to detect PD in its early stage when no visible symptoms are yet observed and no irreversible brain damage occurred. We are also facing a problem of many possible culprits of the disease, among which alpha-synuclein (αSN) is a leading target for medical intervention.
αSN is a natively unfolded presynaptic protein with unknown function in the cell. It can aggregate into stable oligomeric forms and amyloids, which are a main component of LBs found in the postmortem brain tissue of PD patients. Most importantly, it is known that abnormalities in αSN handling are present in PD patients' brains long before neuronal cell loss, what makes it even more interesting target for further research.
CSF is in direct contact with the extracellular space of the brain and it is commonly believed that what is happening in the brain is reflected in the CSF. Subsequently, it is extensively investigated in PD to search for possible biomarkers of early disorder. It has been previously shown that αSN can be found in CSF samples obtained from PD patients. However, the findings are not conclusive and contrary results are presented by different laboratories. Moreover, they are mostly based on total αSN molecules count and therefore do not consider changes in different aggregated species present in CSF.
In our laboratory we have established a new method for detection of αSN aggregates in CSF. Our preliminary data suggest presence of a high molecular weight species, which we are now investigating. We are also testing if with our method we can distinguish control, healthy subjects

from PD cases and if the levels of αSN aggregate changes with severity of disease. Finally, we are also looking for potential αSN interactions in CSF.
We believe that our findings will greatly complement currently available methods for αSN detection and might in the future be useful as a clinical method for assessing PD risk or even confirm occurrence of early stage of this disorder.

Disclosures: W.P. Paslawski: None. **X. Zhang:** None. **P. Svenningsson:** None.

Poster

700. Parkinson's Disease: Biomarkers and Therapeutics

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MOST 104-2314-B-182A-035-

EMRP1E1641

CMRPG3C1482

CMRPG3C0783

CMRPG3C1492

CMRPG3D0382

Title: Increased Rab35 expression is a potential biomarker of Parkinson's disease and implicated in the pathogenesis

Authors: *C.-C. CHIU[1,2], T.-H. YEH[3], Y.-Z. HUANG[5], Y.-J. CHEN[5], C.-L. CHEN[5], H.-L. WANG[6], C.-S. LU[4];
[1]Chang Gung Mem. Hosp., TAOYUAN, Taiwan; [2]Neurosci. Res. Center, Chang Gung Mem. Hosp. at Linkou, Neurosci. Res. Center, Chang Gung Mem. Hosp. at Linkou, Taoyuan, Taiwan; [3]Section of Movement Disorders, Dept. of Neurology, Chang Gung Mem. Hosp. at Linkou Med. Ctr., Taoyuan, Taiwan; [4]Section of Movement Disorders, Dept. of Neurology, Chang Gung Mem. Hosp. at Linkou Med. Ctr., TAOYUAN, Taiwan; [5]Neurosci. Res. Center, Chang Gung Mem. Hosp. at Linkou Med. Ctr., TAOYUAN, Taiwan; [6]Dept. of Physiol. and Pharmacology, Chang Gung Univ. Sch. of Med., Taoyuan, Taiwan

Abstract: Parkinson's disease (PD) is the second common neurodegenerative disease. It is crucial to identify reliable and robust biomarkers for early diagnosis and prediction of disease progression. In order to investigate the alterations of serum protein levels in PD patients, two-dimensional fluorescence differential gel electrophoresis was used to identify differentially expressed serum proteins that might serve as potential biomarkers for PD. The comparative proteomic study of serum samples followed by ELISA confirmation identified that protein expression of Rab35 was upregulated in PD patients (n=213) compared with matched control subjects (n=177) and other parkinsonian disorders, progressive supranuclear palsy (PSP, n=46) and multiple system atrophy (MSA, n=80). The serum level of Rab35 was negatively correlated with the age at onset of PD. There was a significantly correlation between the expression of Rab35 and disease duration of PD. Importantly, PD patients with a high serum level of Rab35 showed shorter overall survival compared to patients with a low expression of Rab35. Moreover, an increased expression of Rab35 protein was observed in the substantia nigra of MPTP-treated mice, rotenone-treated mice, (R1441C) LRRK2 or (G2019S) LRRK2 PD transgenic mice. Furthermore, overexpression of Rab35 significantly increased the aggregation and secretion of A53T α-synuclein in dopaminergic SH-SY5Y cells. Co-expression of Rab35 with wild-type or A53T α-synuclein in SH-SY5Y cells deteriorated cell death. Our results suggest that Rab35 is potentially useful in the differential diagnosis of parkinsonian disorders and is implicated in the pathogenesis of PD.

Disclosures: **C. Chiu:** None. **T. Yeh:** None. **Y. Huang:** None. **Y. Chen:** None. **C. Chen:** None. **H. Wang:** None. **C. Lu:** None.

Poster

700. Parkinson's Disease: Biomarkers and Therapeutics

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 700.09/W8

Topic: C.03. Parkinson's Disease

Title: Frontal dysfunction of Parkinson's disease

Authors: *M. KANAI;
NHO Takasaki Gen. Med. Ctr., Takasaki-City, Gunma, Japan

Abstract: [OBJECTIVE] As a non-motor symptom of Parkinson's disease (PD), cognitive decline and executive dysfunction are pointed out. In comparison with a decrease of memory function, I experience the case that a frontal lobe dysfunction is seen in early. As PD symptoms, I pay my attention to a frontal lobe sign and examine it. [METHOD] I intended for 30 PD patients (a man, a woman each 15) who were under the medical treatment in my hospital. After

having obtained its consent from a patient for the case not to be seen of the memory disorder, I inspected the neuropsychiatric test by clinical psychologists. The inspection battery went mini-mental state examination (MMSE), frontal assessment battery (FAB), trail making test (TMT) and the tower of Hanoi task (TOH). About MMSE and FAB, I added evaluation about the lower item. TMT performed part A and B and evaluated the time required. TOH performed by for each three times with three disks and four disks, and examined it in the shortest achievement time. I set 24 points in a cut-off level about having failure of memory or not in MMSE. [RESULT] 30 all cases were more than 24 points in MMSE. After examining it in a lower item, a drop was seen by attention in MMSE. The conflicting instructions and the prehension behavior were kept in FAB, but a mistake was outstanding in the other subtests. Extension of the time was seen in TMT in complicated part B in comparison with part A. The tendency that time extended to with aging at three disks was seen in TOH, and the case that could not be accomplished with four disks to reflect an executive dysfunction more was seen. FAB score was significant correlation with TMT part B and TOH, but it had no correlation with MMSE score. [CONCLUSION] In the case not to be seen of the cognitive impairment in PD, the deterioration of an attention disorder and the executive dysfunction is accepted. I think that there is the need to diagnose the disorder that is not caught early in MMSE. I provide treatment not only for the motor symptom of PD but also for the frontal lobe dysfunction, under the medical treatment. I think TOH to be effective tools to find out the condition of early stage in the executive dysfunction of PD.

Disclosures: M. Kanai: None.

Poster

700. Parkinson's Disease: Biomarkers and Therapeutics

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 700.10/W9

Topic: C.03. Parkinson’s Disease

Support: National Parkinson Foundation

Don Roberto Gonzalez Family Foundation

Title: Exercise targeting cognitive impairment in Parkinson's disease

Authors: *L. HAWTHORNE[1], M. E. GOMEZ[1], V. FILOTEO[4], A. PETKUS[2], B. JARRAHI[5], I. KUO[3], E. SY[1], S. MCEWEN[5], B. FISHER[3], G. M. PETZINGER[1];
[1]Neurol., [2]Psychology, [3]Div. of Biokinesiology and Physical Therapy, USC, Los Angeles, CA; [4]Psychiatry, Univ. of California San Diego, San Diego, CA; [5]Psychiatry and Behavioral Sci., Univ. of California Los Angeles, Los Angeles, CA

Abstract: Mild cognitive impairment, particularly of the executive function (EF) subtype, is common in Parkinson's Disease (PD), frequently transitions to dementia, and there are no effective interventions to alleviate or slow these cognitive declines. EF processes are needed to learn and optimize performance of complex cognitive and motor skills. Our studies in PD demonstrate that exercise facilitates neuroplasticity of the basal ganglia, supporting the hypothesis that exercise will reverse EF deficits in PD. In addition, our translational animal studies and recent studies in aging and exercise have suggested that skill-based exercise may facilitate cognitive circuitry to a greater extent than aerobic exercise. The aim of this study is to compare and elucidate the effects of skill-based exercise versus aerobic exercise versus control on mild cognitive impairment in Parkinson's disease. Patients will be randomized to 1 of 3 groups (N=20/group) to participate in a community-based neuro-physical therapy guided intervention that will involve either:(i) skill-based exercise; (ii) aerobic exercise or (iii) social-engagement (control) for 36 hours across 12 weeks. Patients assigned to either exercise group will complete 3 one-hour, individual sessions per week in a neurologic physical therapy practice. Blinded evaluators will conduct all assessments at pre-intervention, post-intervention and 3-month follow up visits. Patients will be examined for changes in neurocognitive measures and changes in the connectivity and function of the brain circuits sub-serving executive function seen during an fMRI scan. These outcomes will be compared to changes in measures of cardiovascular and motor/skill-related fitness and body mass index (BMI). Findings from this clinical study could help elucidate the type (skilled vs. aerobic) of exercise that may be most beneficial for improving cognitive function in PD-MCI and the role of exercise in facilitating neuroplasticity (brain connectivity) and repair.

Disclosures: L. Hawthorne: None. **M.E. Gomez:** None. **V. Filoteo:** None. **A. Petkus:** None. **B. Jarrahi:** None. **I. Kuo:** None. **E. Sy:** None. **S. McEwen:** None. **B. Fisher:** None. **G.M. Petzinger:** F. Consulting Fees (e.g., advisory boards); US World Meds.

Poster

700. Parkinson's Disease: Biomarkers and Therapeutics

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 700.11/W10

Topic: C.03. Parkinson's Disease

Support: Michael J. Fox Foundation

Title: Measuring alpha synuclein protein turnover kinetics in cerebrospinal fluid via immunoprecipitation tandem mass spectrometry

Authors: *K. L. PAUMIER, J. G. BOLLINGER, R. MILLER, J. JOCKEL-BALSAROTTI, B. W. PATTERSON, T. M. MILLER, R. J. MILLER, P. T. KOTZBAUER;
Dept. of Neurol., Washington Univ., Saint Louis, MO

Abstract: Parkinson's Disease (**PD**) is defined by the accumulation of alpha-synuclein (**aSyn**) fibrils in neuronal cytoplasmic and neuritic inclusions known as Lewy bodies and Lewy neurites (Forno, 1996). Multiple therapeutic approaches targeting aSyn accumulation are being pursued for the treatment of PD including: 1) inhibition of aSyn protein synthesis, 2) prevention or reversal of aSyn aggregation, and 3) enhancement of aSyn clearance. Hindering the overall development of these therapeutic interventions is the lack of robust methodologies that assess aSyn target engagement and pharmacodynamics. To address this need, we developed an immunoprecipitation tandem mass spectrometry (**IP-LC/MS**) method based on the Stable Isotope Labeling Kinetics (**SILK**) technique to quantitate aSyn and measure its turnover kinetics in human cerebrospinal fluid (**CSF**). SILK has already been successfully applied to measure the turnover of other disease-related proteins in the central nervous system (**CNS**), including amyloid-beta (Bateman et al., 2006, Potter et al., 2013), soluble Amyloid Precursor Protein (Cook et al., 2010, Basak et al., 2012), Apolipoprotein E (Basak et al., 2012, Wildsmith et al., 2012), and superoxide dismutase 1 (Crisp et al., 2015). These studies provide valuable insight into CNS disease mechanisms (Mawuenyega et al., 2010, Potter et al., 2013) as well as in measuring the pharmacodynamic action of proposed disease-modifying drugs (Dobrowolska et al., 2014). Leveraging our previous experience with the development of SILK protocols and monoclonal antibodies, we first developed an IP protocol by screening a panel of both in-house and commercial antibodies under a set of commonly applied IP conditions. We then optimized a LC/MS method based on bottom-up proteomics capable of characterizing the aSyn proteoforms present in human CSF. Using a set of 15-Nitrogen uniformly labeled proteins in addition to a set of peptides with differentially labeled individual amino acids, we designed our quantitative assay around five key proteolytic peptides containing either a Leucine residue (for SILK turnover analysis) or sites of documented post-translational modifications. With optimized assay parameters in hand, we will define a suitable labeling strategy for SILK analysis to determine the production and clearance rates of aSyn in CSF from a set of control human participants and corresponding PD patients. We hypothesize that measurement of aSyn production and clearance rates by SILK will define altered aSyn metabolism related to PD. Further, this technique will provide a valuable measure of target engagement and pharmacodynamics for future clinical studies targeting aSyn.

Disclosures: K.L. Paumier: None. **J.G. Bollinger:** None. **R. Miller:** None. **J. Jockel-Balsarotti:** None. **B.W. Patterson:** None. **T.M. Miller:** None. **R.J. Miller:** A. Employment/Salary (full or part-time): Washington University. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; Brighfocus Foundation, Cure Alzheimer's Fund, Glen Foundation for Medical Research, Metropolitian Life Foundation, NIH/NINDS 2R01NS065667-05, NIH/NIA 5K23AG030946, Pharma Consortium (Amgen, AstraZeneca, Biogen Idec, Eisai, Eli Lilly and Co., FORUM, Hoffman La-Roche Inc., Pfizer Biotherapeutics R and D, Sanofi-

Aventi), Ruth K. Broadman Biomedical Research Foundation, NIH/NIA U19AG32438, NIH RR00954, Biogen/Abbvie. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Alzheimer's Association, Anonymous Foundation, Avid Radiopharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); C2N Diagnostics. F. Consulting Fees (e.g., advisory boards); IMI, Global Alzheimer's Platform, FORUM, OECD, Roche, Boehringer Ingelheim, Merck. **P.T. Kotzbauer:** None.

Poster

700. Parkinson's Disease: Biomarkers and Therapeutics

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 700.12/W11

Topic: C.03. Parkinson’s Disease

Support: NIH R21

MJFF

Title: Do pathogenic LRRK2 mutations result in altered immune cell homeostasis?

Authors: *A. F. CINTRON[1], C. HESS[3], S. ISAACSON[4], C. SINGER[5], T. ZESIEWICZ[6], J. BOSS[2], M. TANSEY[1];
[1]Physiol., [2]Emory Univ., Atlanta, GA; [3]Univ. of Florida, Gainesville, FL; [4]Parkinson's Ctr., Boca Raton, FL; [5]Univ. of Miami, Miami, FL; [6]Univ. of South Florida, Tampa, FL

Abstract: The identification of the leucine-rich repeat kinase 2 (LRRK2) mutation as causative in dominantly inherited form of Parkinson’s disease, led to a new target for investigation in PD. Both idiopathic PD and LRRK2-associated PD have similar clinical presentation and the study of LRRK2 function could shed light onto disease mechanisms and progression which could result in improved therapeutics. LRRK2 mutations are associated with roughly 1-2% of total PD cases and an incidence of 40% in the Ashkenazi Jewish PD population. LRRK2 is abundantly expressed in cells of the immune system, including CD4+ and CD8+ T cells, CD14+ monocytes, and CD19+ B cells; yet its function in the immune system and the relationship of LRRK2 function in immune cells to PD pathogenesis is largely unknown. In this study, we use flow cytometry to characterize immune cell populations in blood of PD patients with and without the LRRK2 mutation as well as healthy controls with and without the mutation. Flow cytometry used with a cocktail of antibodies enables us to identify monocytes, B cells, and T-cells including central and effector memory populations as well as Th_1, Th_2, Th_{17}, and T_{reg} populations. These studies are expected to show alterations in activation responses in immune

cell populations as a result of a pathogenic LRRK2 mutation. This study can provide the first evidence of immune effector cell differences between pathogenic LRRK2 mutations and controls.

Disclosures: **A.F. Cintron:** None. **C. Hess:** None. **S. Isaacson:** None. **C. Singer:** None. **T. Zesiewicz:** None. **J. Boss:** None. **M. Tansey:** None.

Poster

700. Parkinson's Disease: Biomarkers and Therapeutics

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 700.13/W12

Topic: C.03. Parkinson's Disease

Title: ^{123}I-Ioflupane SPECT predicts the efficacy of selegiline monotherapy for motor symptoms in drug-naïve Parkinson's disease

Authors: *H. MURAKAMI[1], S. ISHIGAKI[2], H. KATOH[3], K. ONO[1];
[1]Dept. of Neurol., Sch. of Medicine, Showa Univ., Shinagawa-ku Tokyo, Japan; [2]Dept. of Neurol., Showa University, Northern Yokohama Hosp., Yokohama, Japan; [3]Dept. of Neurol., Showa Univ. Fujigaoka Hosp., Yokohama, Japan

Abstract: (Background) Selegiline, a monoamine oxidase inhibitor, enhances the patient's own endogenous dopamine in their brain by inhibiting the metabolism of dopamine. Dopamine improves Parkinsonian symptoms. Thus, efficacy of selegiline monotherapy for de novo Parkinson's Disease (PD) patients is speculated to depend on residual nigrostriatal dopaminergic neurons. ^{123}I-Ioflupane single photon emission computed tomography (SPECT) is a method to assess striatal degeneration. (Aim) We examined the utility of ^{123}I-Ioflupane SPECT to predict the efficacy of selegiline monotherapy for motor symptoms in drug-naïve PD patients. (Methods) Nineteen drug-naïve, de novo PD patients (6 males, 13 females) began taking selegiline monotherapy, and the dose was increased until improved
motor function without side effects (maximum 10 mg per day) was obtained. Motor function at baseline and after the medication had reached the stable dose was assessed using the Unified Parkinson's Rating Scale (UPDRS). ^{123}I-Ioflupane SPECT was performed before the start of the medication, and the Specific Binding Ratio (SBR) in the striatum was calculated for each participant. The SBR and improvement in motor assessment score were compared using Spearman's correlation coefficient. (Results) The UPDRS part III (motor score) at baseline did not correlate with the SBR in the stratum ($p=0.19$). Improvement from baseline in the UPDRS part III showed a significant negative correlation with the SBR in the striatum ($p<0.01$). In other words, patients with a low SBR had grater improvement with the selegiline monotherapy than

those patients with a higher SBR. Multiple regression analysis taking into account the age of the patient and the selegiline dose showed that improvement in the UPDRS part III significantly correlated with the SBR ($p<0.05$), but it did not correlate with the age of the patient ($p=0.45$) and the drug dose ($p=0.28$). (Conclusion) ^{123}I-Ioflupane SPECT can predict the efficacy of selegiline monotherapy when used as an initial treatment for drug-naïve PD patients.

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Poster

700. Parkinson's Disease: Biomarkers and Therapeutics

Location: Halls B-H

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Topic: C.03. Parkinson’s Disease

Support: Gifts to the Brain Restoration Center

Tom Dupree for Parkinson's Disease Research

University of Kentucky start-up funds

National Center for Advancing Translational Sciences grant UL1TR000117

Title: Microscopic, biochemical, and immunohistochemical characterization of peripheral nerve tissue used in brain autografts for the treatment of Parkinson's disease

Authors: *A. S. WELLEFORD[1,2], C. G. VAN HORNE[1,2], J. E. QUINTERO[1,2], Y. AI[1], G. A. GERHARDT[1,2];
[1]Univ. of Kentucky, Lexington, KY; [2]Brain Restoration Ctr., Lexington, KY

Abstract: Currently two clinical trials (NCT01833364 and NCT02369003) are underway which feature the implantation of a peripheral nerve autograft to the brain (targeted either to the Substantia Nigra or the Nucleus Basalis of Meynert) in combination with Deep Brain Stimulation (DBS) for the treatment of patients with Parkinson’s disease. This nerve tissue is harvested from the sural nerve, a cutaneous sensory nerve located in the lateral ankle, of patients undergoing DBS surgery. Two tissue samples per patient are collected for study (one during the Stage I surgery, another during the Stage II surgery 5-7 days later) in addition to the tissue used for the graft. As of 5/1/16, 28 patients have received a graft.
The character of the peripheral nerve tissue used in these clinical trials has yet to be described. This study examines several aspects of the peripheral nerve tissue; including microscopic appearance, levels of neurotrophic factors, morphology of Schwann Cells, and presence of

macrophages. These results are supplemented by immunohistochemical analysis of the brain of non-human primates that have undergone an analogous procedure. The results of this model show growth of tyrosine hydroxylase-containing nerve fibers, which are a marker of dopamine-producing neurons, into the area of the peripheral nerve graft. In addition, results in this model show the presence of S100beta-containing cells as well as GFAP-containing cells within and surrounding the graft.

Disclosures: **A.S. Welleford:** None. **C.G. van Horne:** Other; Educational support grant through Medtronic. **J.E. Quintero:** None. **Y. Ai:** None. **G.A. Gerhardt:** None.

Poster

700. Parkinson's Disease: Biomarkers and Therapeutics

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Program#/Poster#: 700.15/X2

Topic: C.03. Parkinson’s Disease

Support: NSF Grant No. 10037840

NSF CAREER Award Grant No. 58501963

NSF GRFP Grant No. 1256065

Title: Optimized programming algorithm for cylindrical and directionally segmented deep brain stimulation electrodes

Authors: ***D. N. ANDERSON**, B. OSTING, A. DORVAL, C. R. BUTSON;
Univ. of Utah, Salt Lake City, UT

Abstract: **Introduction:** Deep brain stimulation (DBS) programming is a complex process likely to become more complex with the introduction of leads with larger numbers of contacts. We developed an automated programming algorithm to optimize DBS parameter selection for targeted neural activation in a patient-specific manner. The purpose of this study is to assess algorithm performance by applying it to conventional and directional electrode geometries. **Methods:** We used finite element models to solve the bioelectric field problem for a conventional 4-contact DBS lead (Medtronic 3387) and three directional leads: the directSTNactute (Pollo et al., 2014), the segmented lead (Bulmann et al., 2011), and the Sapiens electrode (Decré et al., 2013). As shown in Figure 1A, a 3D rendering of the stimulation target area and fiber tractography were generated from patient MRI and diffusion tensor imaging (DTI). We used values derived from the Hessian matrix of voltage second derivatives as an estimate of neural activation to maximize stimulation in the STN and limit activation of axon

fibers in the internal capsule. **Results:** We have demonstrated an ability to program the electrode to stimulate a target area while avoiding neural tracts that may be responsible for side effects. We tested that the algorithm settings were robust by adjusting the magnitude of optimized electrode settings as shown in Figure 1B. Optimal parameter settings from the algorithm show STN activation while limiting stimulation outside the target area and the internal capsule. **Conclusion:** We have developed a method of optimization that can be applied to the clinical electrode and additional complex DBS lead designs. Deviation away from the optimized parameters showed more stimulation outside the target area or activation of neighboring fiber tracts. A real-time, patient-specific, automated programming algorithm may increase efficiency and positive outcomes of clinical DBS programming, as well as enable the use of more complex lead designs, which are likely to be too complex for manual programming.

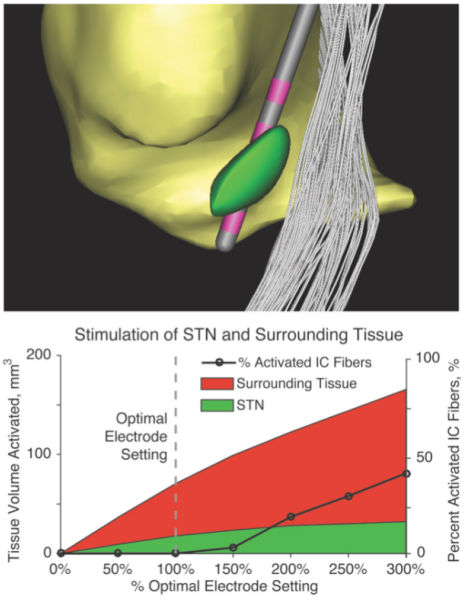

Figure 1. **A.** The neural target for our study is the subthalamic nucleus (STN), in green, a common surgical target for DBS for Parkinson's disease. Stimulation of the internal capsule (IC), a white matter tract adjacent to the STN, is associated with unwanted sensorimotor side effects, and will be used as an area of avoidance in our algorithm (Xu et al., 2011). **B.** We compare activated volume of the STN, surrounding tissue, and percent activation of internal capsule tracts to verify optimized settings for the conventional electrode. Optimal settings using a neural activation threshold of 8 mV were calculated to be 0.3 V and 0.8 V for contacts 0 and 1, respectively. As applied voltage increased, more surrounding tissue and IC fibers were activated, while lower settings did not activate as much of the STN volume.

Disclosures: **D.N. Anderson:** None. **B. Osting:** None. **A. Dorval:** None. **C.R. Butson:** 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; NSF 10037840. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Intelect Medical. F. Consulting Fees (e.g., advisory boards); St Jude Medical, Functional Neuromodulation.

Poster

700. Parkinson's Disease: Biomarkers and Therapeutics

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Program#/Poster#: 700.16/X3

Topic: C.03. Parkinson's Disease

Support: NSF-IGERT Program (DGE-1069104)

National Science Foundation Graduate Research Program

Michael J. Fox Foundation and NIH (R01-NS081118)

Title: Spatial and geometric performance of particle swarm optimization (PSO) for programming deep brain stimulation arrays

Authors: ***E. PEÑA**[1], S. ZHANG[2], Y. XIAO[2], M. JOHNSON[2];
[1]Biomed. Engin., Univ. of Minnesota Twin Cities, Saint Paul, MN; [2]Univ. of Minnesota, Twin Cities, Minneapolis, MN

Abstract: Deep brain stimulation (DBS) therapy relies on both precise neurosurgical targeting and systematic optimization of stimulation settings to achieve beneficial clinical outcomes. One recent advance to improve targeting is the development of DBS arrays (DBSAs) with electrodes segmented both along and around the DBS lead. However, increasing the number of independent electrodes creates the logistical challenge of optimizing stimulation parameters efficiently. We developed a robust and efficient particle swarm optimization (PSO) approach to solve this complex multi-objective programming problem through a swarm of individual particles representing a range of electrode configurations and stimulation amplitudes. The approach was evaluated across several axonal fiber orientations relative to the DBSA lead (0°, 30°, 60°, 90°, 120°, and 150°). The fiber tracts consisted of a region of interest (ROI) and a region of avoidance (ROA). Accuracy of PSO activation predictions was assessed using biophysical NEURON models. To investigate the specificity of PSO, we generated complex, angled fiber tracts with an

ROI and ROA. The lead was then shifted relative to the fiber tracts. For all fiber tracts, finite element models were used to determine the nodal voltages for each electrode configuration. The PSO algorithm solved the multi-objective ROI-ROA problem by producing consistent Pareto fronts. The angle of fibers was found to influence the activation threshold needed for the PSO output to match the biophysical model predictions. The specificity of the PSO algorithm was maintained across DBSA lead shifts, such that activation in the ROI was always 25 to 100 times higher than in the ROA. These results suggest that if one accounts for the axonal orientations around a DBS lead, the PSO algorithm can provide a computationally efficient way to program DBS systems especially those with higher electrode counts.

Disclosures: E. Peña: None. **S. Zhang:** None. **Y. Xiao:** None. **M. Johnson:** None.

Poster

700. Parkinson's Disease: Biomarkers and Therapeutics

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 700.17/X4

Topic: C.03. Parkinson's Disease

Title: Deep brain stimulation Activa SC neurostimulator battery longevity; initial clinical data

Authors: *R. P. PATEL[1], R. J. DIPAOLA[2], S. F. DANISH[1], S. WONG[2], E. L. HARGREAVES[1];
[1]Div. of Neurosurg., [2]Dept. of Neurol., Rutgers Robert Wood Johnson Med. Sch., New Brunswick, NJ

Abstract: Deep Brain Stimulation (DBS) is an adjunct neurosurgical treatment for Movement Disorders. At the core of the DBS system is the neuromodulation device. Medtronic's most recent Activa (SC) neuromodulation device became available 3/15/2011, and purports to have a 2-5 year battery life. We reviewed the charts of 27 individuals implanted with 50 Activa SCs between 3/15/2011 and 9/24/2013. Twenty of the individuals were diagnosed with Parkinson's Disease, 18 Subthalimic Nucleus, 2 Globus Pallidus interna (GPi), two were diagnosed with Dystonia, 2 GPi, 4 with Essential Tremor, 4 Ventral Intermediate nucleus of the thalamus (VIM), and one individual with Multiple Sclerosis tremor 1 VIM. Of the implanted Activa SCs, 23/50 have been exchanged, while 16/50 continue to function, while the final 11/50 have been lost to follow up. The mean start value of the 50 devices was 3.11V, with a range of 3.04V-3.17V. The mean end value of the 23/50 exchanged devices were split into those that passed below the Elective Replacement Indicator (ERI) threshold (n=16, mean 2.50V, sem .03; range 2.22V-2.60V) and those that were tied to a below threshold device and exchanged at the same time (n=7, mean 2.80V, sem .02; range 2.71V-2.86V). The 16/23 exchanged devices from 15

individuals had a mean duration 2.87 years ranging from 1.75 years to 3.98 years, while the ongoing 16/50 devices from 9 individuals currently have a mean duration of 3.3 years ranging from 2.61 years to 4.18 years. Both these values may underestimate the eventual longevity as the devices have only been available for 5.20 years. Of the 16 Activa SCs exchanged at or below the ERI 2.60V threshold, full battery tracking data were available for 10 of the devices. Examination of the plotted battery charge decay curves suggests a cubic spline best fit with an initial concave up followed by a concave down, and a central plateau in between. The first upward concavity is characterized by a rapid decline from the respective start values to a leveling off value ranging from 2.95V to 2.98V occurring by the end of the initial six months. The central plateau occurs between the battery charge values of 2.95V to 2.88V, lasting from 6 months to 3 years dependent upon programmed neuromodulation parameters. The second downward concavity declines more rapidly as the battery runs down with durations of approximately 6 months. The initial upward concavity was fairly homogenous across curves, while the downward concavity was fairly heterogeneous, fanning out to cross the 2.60V ERI threshold at different durations. We believe that the battery duration will continue to grow as the window from the initial implant expands to encompass 5 years from our last implant inclusion.

Disclosures: **R.P. Patel:** None. **R.J. DiPaola:** None. **S.F. Danish:** None. **S. Wong:** None. **E.L. Hargreaves:** None.

Poster

700. Parkinson's Disease: Biomarkers and Therapeutics

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Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 700.18/X5

Topic: C.03. Parkinson’s Disease

Support: NIH CTSA-UL1TR00017

Title: DBS Plus: combining deep brain stimulation and autologous peripheral nerve grafts as an approach to treat motor and cognitive symptoms of Parkinson's disease

Authors: *C. G. VAN HORNE, G. QUINTERO, J. GURWELL, A. ANDERSON, A. WELLEFORD, J. R. LAMM, J. SLEVIN, G. GERHARDT, G. GERHARDT;
Dept Neurosurg, Univ. of Kentucky, Lexington, KY

Abstract: Introduction: We are investigating a strategy that couples a biological therapy with DBS surgery in an attempt to restore areas of the brain affected in PD. We grafted autologous Schwann cells from the sural nerve, known to release a host of growth factors including GDNF, NGF, BDNF, and NT-3. We describe our ongoing clinical trials examining the safety and

feasibility of implanting autologous peripheral nerve grafts in PD in patients undergoing DBS surgery.
Methods: DBS surgery targeting the subthalamic nucleus or internal globus pallidus was performed using standard procedures. A 5mm section of sural nerve was excised, stripped of the epineurium, cut into 1mm pieces, and unilaterally delivered into the area of the substantia nigra (SN) or nucleus basalis of Meynert (NBM). Adverse events were continuously monitored. Assessments included neurocognitive performance, quality of life, gait, (123I-ioflupane) SPECT imaging, and MR imaging at baseline and at the end of the study, 24 months after the implant surgery. Subjects undergo a Unified Parkinson's Disease Rating Scale (UPDRS) evaluation before surgery and at 6, 12, 18 and 24 months after surgery.
Results: We recently completed a 1-year Phase I study (NCT01833364) of 8 participants and found an adverse event profile comparable to standard DBS surgery. In addition, 6 of 8 participants showed a lower UPDRS III motor score than at baseline (25.5 ± 16.8 at 12 months vs. 32.5 ± 9.8 at baseline; Mean ± SD, N=8) considered a moderate clinically important difference (Shulman et al. 2010). This compares to 12 PD patients with bilateral DBS alone at 12 months showing a 5 point increase (60.7 ± 14.1 at 12 months vs. 55.7 ± 17.4). A follow-up, open-label, two-year study of safety and feasibility (NCT02369003) has been initiated that allows DBS of the GPi. Mean age for participants was 66.6 ± 6.9, Hoehn & Yahr score: 3.2 ± 0.5, UPDRSIII OFF medication: 37.7 ± 9.5, and UPDRSIII ON medication: 18.3 ± 10.5. One year data for this group will be presented. Participants showed a decrease in striatal 123I-ioflupane binding with SPECT imaging. Overall, we have successfully completed peripheral nerve graft delivery to the SN in 14 participants, and to the NBM in 6 participants in this study. Immediate post-operative MRIs did not indicate evidence of abnormal tissue disruption. There have been no serious adverse events related to graft placement.
Conclusions: Initial results from two clinical trials indicate a potentially safe and feasible means of delivering biological therapy with DBS surgery. With respect to motor symptoms, participants with grafts have reduced UPDRS III scores compared to baseline and to controls, who showed and increase in their score.

Disclosures: C.G. van Horne: None. **G. Quintero:** None. **J. Gurwell:** None. **A. Anderson:** None. **A. Welleford:** None. **J.R. Lamm:** None. **J. Slevin:** None. **G. Gerhardt:** None. **G. Gerhardt:** None.

Poster

700. Parkinson's Disease: Biomarkers and Therapeutics

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 700.19/X6

Topic: C.03. Parkinson's Disease

Support: Parkinson Society Canada Graduate Award (JFD)

Fonds de Recherche du Québec-Santé (JFD)

Natural Sciences and Engineering Research Council of Canada operating grant (CD)

Title: Dopaminergic medication and subthalamic deep brain stimulation do not improve inter-limb coupling in Parkinson's disease

Authors: *J.-F. DANEAULT[1,2], B. CARIGNAN[3], A. SADIKOT[2], C. DUVAL[3];
[1]Physical Med. and Rehabil., Harvard Med. Sch., Charlestown, MA; [2]McGill Univ., Montreal, QC, Canada; [3]Univ. du Québec à Montréal, Montréal, QC, Canada

Abstract: INTRODUCTION: Parkinson's disease (PD) is a progressive neurodegenerative disorder that leads to severe motor and non-motor impairments. The motor impairments are believed to be caused by aberrant neuroplastic changes in the basal ganglia as well as primary and accessory motor networks. We have previously demonstrated that inter-limb coupling is altered in patients with PD. Inter-limb coupling is a specific feature of bimanual tasks characterized by the harmonization of movement parameters between limbs. OBJECTIVES: The goal of the current study was to identify the impact of subthalamic deep brain stimulation (DBS) and dopaminergic medication on inter-limb coupling. METHODS: Twenty patients with PD were first tested approximately one week before subthalamic DBS surgery. After surgery, when their motor state was deemed stable by their treating physician, patients were again assessed. Pre- and post-surgery assessments were performed on and off medication. Post-surgery assessments were also performed on and off DBS. Patients were asked to perform rapid repetitive pronation-supination movements unimanually and bimanually. The difference in mean amplitude and mean duration of cycles between hands was computed in order to assess spatial and temporal inter-limb coupling, respectively. Structural coupling between hands during the bimanual task was also computed. RESULTS: Pre-surgery off medication data replicated our previous data showing that PD patients exhibit temporal inter-limb coupling but not spatial inter-limb coupling. Results of the current study demonstrate that the temporal inter-limb coupling exhibited by patients with PD is not altered by either dopaminergic medication or subthalamic DBS. This indicates that temporal inter-limb coupling is likely mediated by structures not affected by PD and that lie outside the networks whose activity is altered through dopaminergic medication or subthalamic DBS. The lack of spatial inter-limb coupling was not corrected by either dopaminergic medication or subthalamic DBS. This indicates that spatial inter-limb coupling is likely mediated by structures that are affected by PD but that lie outside the networks whose activity is altered through dopaminergic medication or subthalamic DBS. CONCLUSION: The current study provides preliminary insight into the possible neural mechanisms mediating inter-limb coupling. This may lead to a better understanding of bimanual coordination as well as the development of novel interventions to improve the performance of bimanual tasks in patients with PD.

Disclosures: **J. Daneault:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neuromotrix. **B.**

Carignan: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neuromotrix. **A. Sadikot:** None. **C. Duval:** None.

Poster

700. Parkinson's Disease: Biomarkers and Therapeutics

Location: Halls B-H

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Program#/Poster#: 700.20/X7

Topic: C.03. Parkinson's Disease

Support: Doris Duke Charitable Foundation Clinical Scientist Development Award (W. F. A)

Title: A low-cost, intraoperative movement quantification system for DBS surgery and neurophysiology

Authors: *E. SCHAEFFER[1], D. LIU[1], M. AHN[1,2], J. A. GUERIN[1,2], S. LEE[1,2], W. F. ASAAD[1,2,3,4,5];
[1]Neurosci., Brown Univ., Providence, RI; [2]Brown Inst. for Brain Sci., Providence, RI; [3]Neurosurg., Brown Univ. Alpert Med. Sch., Providence, RI; [4]Neurosurg., Rhode Island Hosp., Providence, RI; [5]Rhode Island Hosp. Norman Prince Neurosciences Inst., Providence, RI

Abstract: Parkinson's Disease (PD) and Essential Tremor (ET) are the two most common movement disorders. In severe, medication-refractory cases, Deep Brain Stimulation (DBS) is often performed as an awake neurosurgical procedure to help alleviate these motor symptoms. During the surgical implantation of DBS electrodes, microelectrode recordings are employed to identify the position of the electrodes as they are advanced toward the predetermined therapeutic target area of the brain. For PD, this target is often the subthalamic nucleus (STN) or the globus pallidus internus (GPi), and for ET the target is the ventral intermediate nucleus of the thalamus (VIM). The somatotopic organization of these target structures is utilized to guide the final placement of DBS electrodes by assessing changes in neural activity in response to passive or active movements of the patients' extremities. Typically, however, the audible detection of such modulations of neural activity is reliant upon the perception of trained observers, which can be a biased assessment. Background noise from both neural and non-neural sources contribute to the inherently qualitative nature of this assessment. Thus, in the intraoperative setting, there is a need for online quantification of synchronized neural activity and movement data to increase the sensitivity and specificity of localizing the final stimulation target. To perform a principled assessment with high temporal precision of neural changes due to movement, we developed a low-cost, Arduino-based Inertial Measurement Unit (IMU) with generalized analog outputs to enable synchronization with our neural data acquisition hardware. We discuss how our system

may help to improve understanding of the relationship between somatotopy and therapeutic benefit, ultimately leading to improved care for PD and ET patients.

Disclosures: **E. Schaeffer:** None. **D. Liu:** None. **M. Ahn:** None. **J.A. Guerin:** None. **S. Lee:** None. **W.F. Asaad:** None.

Poster

700. Parkinson's Disease: Biomarkers and Therapeutics

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 700.21/X8

Topic: C.03. Parkinson's Disease

Support: Rhode Island Neuroscience Collaborative

Title: Impact of dbs-stn on speech for people with parkinson disease impact of dbs-stn on speech for people with parkinson disease impact of dbs-stn on speech for people with parkinson disease

Authors: *L. A. MAHLER[1], D. RYDER[2];
[1]Interdisciplinary Neuroscience/Communicative Disorders, [2]Interdisciplinary Neurosci. Program, Univ. of Rhode Island, Kingston, RI

Abstract: *Objective:* The purpose of this study was to describe speech characteristics of two adults with idiopathic Parkinson disease before and after receiving deep brain stimulation of the subthalamic nucleus (DBS). *Methods:* A single-subject A-B-A-A research design was used to detect the impact of DBS on characteristics of speech and voice. Evaluations were obtained at three time points; pre-surgery, post-surgery, and follow-up. DBS01 was a 53-year-old male two years post diagnosis. His speech characteristics were consistent with hypokinetic dysarthria including reduced speech intensity, mildly imprecise articulation, and monotone pitch. He received bilateral electrode placements in the subthalamic nucleus. DBS02 was a 58-year-old male five years post-diagnosis. He had hypokinetic dysarthria characterized by reduced speech intensity, breathy voice, monotone pitch, and mildly imprecise articulation. He received unilateral electrode placement in the left subthalamic nucleus. The dependent variables included: intelligibility for words and sentences, speech intensity (dB SPL), and vowel space area. *Results:* There was a statistically significant decrease ($p=0.01$) in single word intelligibility for DBS01 from 86.8% (11.2) pre-surgery to 70.4% (4.6) at follow-up. Sentence intelligibility remained unchanged. There was a statistically significant increase ($p=0.02$) in single word intelligibility for DBS02 from 79.6 (6.2) to 90.4 (3.8) post-surgery that returned to baseline at follow-up. Sentence intelligibility remained unchanged. Both participants read words and sentences at reduced intensity and this did not change following surgery. Average loudness for DBS01 during

word reading was 58.9 (1.9) pre-surgery and 58.1 (2.9) post-surgery and for DBS02 was 59.8 (3.2) pre-surgery and 59.2 (3.0) post-surgery compared with average normal loudness for a male of 72.0 dB SPL measured at 40 cm. Vowel space area decreased for DBS01 from 199,000 Hz^2 pre-surgery to 175,000 Hz^2 at follow-up and increased for DBS02 from 170.000 Hz^2 pre-surgery to 218,000 Hz^2 at follow-up. *Conclusions:* DBS01 received bilateral DBS and his results showed a decline in single word intelligibility following surgery and a compression of vowel space area associated with less precise articulation. DBS02 received unilateral DBS and his results showed an increase in single word intelligibility and an expansion of vowel space area associated with greater precision of articulation. The variability of responses in these two participants indicates that further research is necessary to understand the impact of DBS on speech, which can affect quality of life for people with PD.

Disclosures: L.A. Mahler: None. **D. Ryder:** None.

Poster

700. Parkinson's Disease: Biomarkers and Therapeutics

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 700.22/X9

Topic: C.03. Parkinson’s Disease

Title: Modeling speech disorders in parkinson’s disease using parsimonious factors

Authors: *I. EL MOUDDEN[1], M. OUZIR[2], B. BENYACOUB[1], S. ELBERNOUSSI[1]; [1]Fac. of Sci. Mohammed V Univ. In Rabat, Rabat, Morocco; [2]Dept. of Biol., Lab. of biochemistry and Immunology, Fac. of Sciences, Mohammed V Univ. in Rabat, Rabat, Morocco

Abstract: Speech disorders represent an early and common manifestation of Parkinson’s disease. The speech impairment relevant to Parkinson’s disease is described as dysphonia (inability to produce normal vocal sounds) and dysarthria (difficulty in pronouncing words). Class prediction is an essential task in automatic speech treatment. Many classification experiments have been performed which focuses on the automatic detection of Parkinson’s disease patients from healthy speakers, but results are still not optimistic. High dimensionality of data is a major problem usually seen in accomplishing this task. A proposed framework to solve this problem is to employ dimension reduction statistical techniques. In this work, the potential of Common factor analysis (CFA), Principal Component Analysis (PCA) based modeling in dimensionality reduction is taken into consideration as the data smoothening tool with multiclass target expression data. After, on the basis of suggested CFA and PCA-based modeling, the power class prediction of logistic regression (LR) and decision tree (DT) in numeric data to develop an advanced classification model is investigated. This framework is applied to a publicly

available Parkinson's disease dataset Silverman voice treatment (LSVT). The main advantage of those approaches is that the number of factor can be effectively reduced from p=309 to k=32 for LSVT Voice Rehabilitation dataset, with a fine classification accuracy of 100% and 99,92% for CFA-LR and PCA-LR respectively. In addition, using only 9 dysphonia features, classification accuracy was (99.20%) and (99.11%) for CFA-DT and PCA-DT respectively. In sum, our combined dimension reduction and data smoothening approaches have significant potential to minimize the number of features and increase the classification accuracy and then automatically classify subjects in Parkinson's disease patients from healthy speakers.
Keywords: Dimension Reduction, Classification, Parkinson's Disease, Neuro-Computation.

Disclosures: I. El Moudden: None. **M. Ouzir:** None. **B. Benyacoub:** None. **S. ElBernoussi:** None.

Poster

700. Parkinson's Disease: Biomarkers and Therapeutics

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 700.23/X10

Topic: C.03. Parkinson's Disease

Support: NIH/NINDS K25 NS053544

NSF/CBET CAREER 1351112

Title: Stimulation of the subthalamic nucleus exacerbates dysarthria in a rat model of Parkinsonism

Authors: *A. D. DORVAL[1], N. O. KING[3], C. J. ANDERSON[2];
[1]Dept. of Bioengineering, [2]Dept. of Neurol., Univ. of Utah, Salt Lake City, UT; [3]Dept. of Biomed. Engin., Washington Univ., St. Louis, MO

Abstract: Deep brain stimulation (DBS) of the subthalamic nucleus typically alleviates most of the primary motor symptoms of parkinson disease (PD) - resting tremor, bradykinesia, and rigidity - but its effect on more centralized symptoms (e.g., postural instability) is less universally therapeutic. Recent work suggests that DBS may worsen medial, secondary motor symptoms including parkinsonian dysarthria, an umbrella term that describes vocal articulation problems resulting in soft, slurred speech, and presumably related to an underlying hypokinesia. Because the mechanisms of DBS-exacerbated dysarthria are poorly understood and difficult to study in patients with PD, we developed a rodent model of DBS-exacerbated dysarthria. We used a standard rat model of DBS-alleviated PD: 6-hydroxydopamine was injected to the medial

forebrain bundle of adult male rats, lesioning dopaminergic neurons in substantia nigra pars compacta; and therapeutic high frequency stimulation was delivered to the subthalamic nucleus. Ultrasonic (20-100 kHz) mating calls were recorded from sexually -experienced and -frustrated males as they were exposed to, but restrained from, female rats in estrus. Vocalization statistics were calculated from mating calls collected under naïve, PD, and DBS-alleviated conditions. Compared to naïve, rats in the PD condition made fewer calls of shorter duration and less complex structure. To the extent that these changes represent aspects of hypokinetic dysarthria, each aspect was exacerbated by putatively therapeutic DBS. Interestingly, individual utterances spanned a wider frequency range in the PD condition compared to naïve, and this effect was also exaggerated by DBS. We propose that together, the changes in vocalization statistics — call recurrence, duration, complexity, and frequency range — constitute a rodent model of parkinsonian dysarthria. Our presentation will include a detailed procedure for vocalization filtering and statistic calculation, as well as analyses of these vocalization statistics in both the low (20-40 kHz) and high (40-100 kHz) frequency call ranges. In future work, this rodent model will enable explorations of the mechanisms of DBS-exacerbated parkinsonian dysarthria, and potentially help modify DBS therapy to alleviate dysarthria and improve other medial symptoms.

Disclosures: **A.D. Dorval:** None. **N.O. King:** None. **C.J. Anderson:** None.

Poster

701. Peripheral Neuropathy

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 701.01/X11

Topic: C.05. Neuromuscular Diseases

Support: Medical Research Council Centre for Neuromuscular Diseases PhD Studentship

National Institute for Health Research

Clinical Research and Development Committee, University College London Hospitals Charities

The Brain Research Trust

Title: Cellular pathomechanisms of Hereditary Sensory Neuropathy type 1 (HSN1) in mammalian motor neurons

Authors: ***E. WILSON**[1,2], B. KALMAR[1], M. KUGATHASAN[1,2], A. ABRAMOV[3], M. M. REILLY[2], L. GREENSMITH[1,2];

[1]Sobell Dept. of Motor Neurosci. and Movement Disorders, [2]MRC Ctr. for Neuromuscular Dis., [3]Dept. of Mol. Neurosci., UCL Inst. of Neurol., London, United Kingdom

Abstract: HSN1 is a peripheral neuropathy most frequently caused by missense mutations in the *SPTLC1* or *SPTLC2* genes, which code for two subunits of the enzyme serine palmitoyltransferase (SPT). SPT catalyzes the first and rate limiting step of *de novo* sphingolipid synthesis. It has been shown that mutations in SPT causes a change in enzyme substrate specificity which results in the production of two atypical sphinganines, deoxysphinganine (DSp) and deoxymethylsphinganine (DMSp), rather than the normal enzyme product, sphinganine (Sp). Levels of DSp and DMSp are elevated in the blood of HSN1 patients and this is thought to cause the peripheral nerve damage characteristic of the disease, which affects both sensory as well as motor axons. However, the underlying pathomechanism of how DSp and DMSp damage neurons remains elusive. In this study DSp and DMSp mediated neurotoxicity was examined in primary mammalian motor neurons by assessing cell survival and neurite outgrowth. These abnormal enzyme products were found to have a rapid, dose dependent, neurotoxic effect in motor neurons. We also explored the potential mechanisms that may underlie DSp and DMSp neurotoxicity and found that mitochondrial dysfunction and calcium handling deficits may be key mediators of abnormal sphingolipid neurotoxicity.

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Poster

701. Peripheral Neuropathy

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 701.02/X12

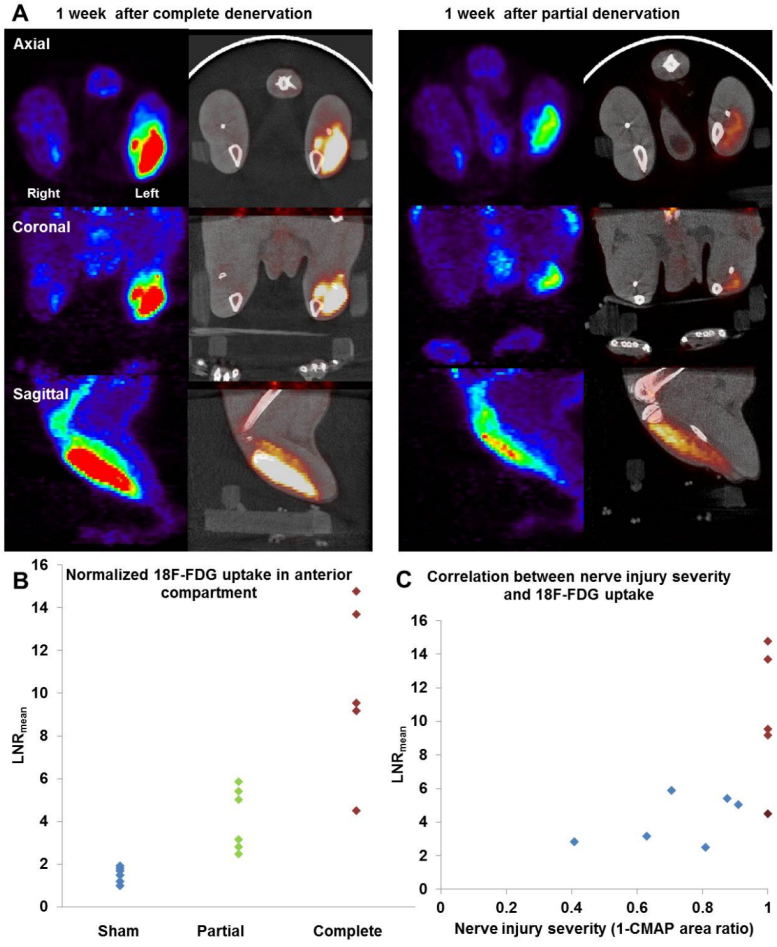
Topic: E.10. Motor Neurons and Muscle

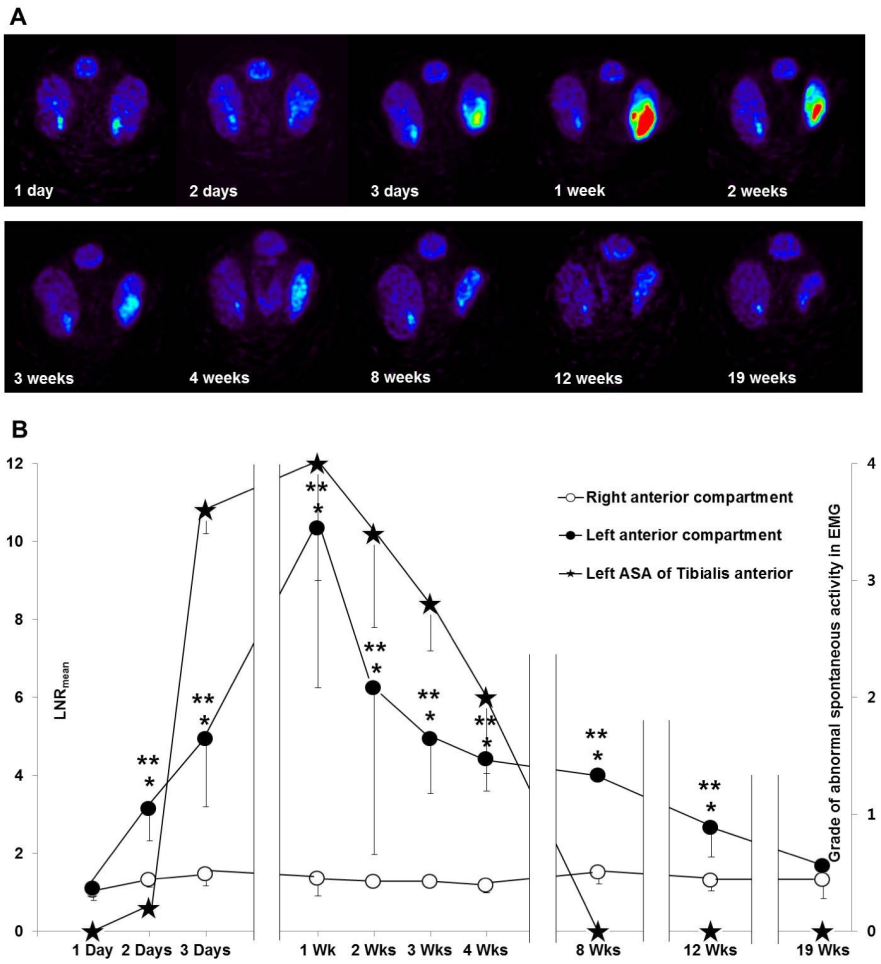
Support: National Research Foundation of Korea (NRF) 2015R1C1A1A02037192

Title: Temporal course and relationship to nerve injury severity of the glucose hypermetabolism in denervated skeletal muscle

Authors: *S. LEE[1], H. SEO[2], B.-M. OH[2], H. CHOI[3], G. CHEON[3,4], S.-U. LEE[5], K. KIM[2]; [1]Dept. of Rehabil. Med., Seoul Natl. Univ. Hosp., Seoul-City, Korea, Republic of; [2]Dept. of Rehabil. Medicine, Seoul Natl. Univ. Hosp., Seoul, Korea, Republic of; [3]Dept. of Nuclear Medicine, Seoul Natl. Univ. Hosp., Seoul, Korea, Republic of; [4]Inst. of Radiation Medicine, Med. Res. Center, Seoul Natl. Univ., Seoul, Korea, Republic of; [5]Dept. of Rehabil. Medicine, Seoul Natl. Univ. Boramae Med. Ctr., Seoul, Korea, Republic of

Abstract: Glucose hypermetabolism in denervated skeletal muscle is a peculiar phenomenon which has been shown by our previous animal study. The purpose of this study is to investigate the relationship between the severity of nerve injury and the signal intensity of l8F-FDG uptake and the temporal course of glucose hypermetabolism in denervated muscles. One week after partial ligation of left peroneal nerve, 18F-FDG PET scans were done and mean lesion-to-normal counts ratios (LNR_{mean}) were calculated for anterolateral compartment regions of interests (ROIs). To examine the temporal course, serial 18F-FDG PET scans and electromyography (EMG) were done in complete sciatic transection with contralateral sham-operation. In the partial peroneal injury model, there was strong positive correlation between electrophysiologically calculated nerve injury severity and LNR_{mean} (Pearson correlation coefficient 0.63, $p<0.03$). Seiral 18F-FDG PET scans in complete injury model showed significantly increased l8F-FDG uptake began at 2 days (LNR_{mean}, left, 3.15 ± 0.82; right, 1.33 ± 0.21, $n = 5$, $P < 0.05$) after denervation and continued to 12 weeks. Fibrillation potentials and positive sharp waves in EMG were abruptly appeared in 3 days and disappeared in 12 weeks after the surgery. Our study demonstrated that the amount of glucose hyperrmetabolism in denervated skeletal muscle is related to nerve injury severity and it has temporal characteristics after nerve injury.





Disclosures: **S. Lee:** None. **H. Seo:** None. **B. Oh:** None. **H. Choi:** None. **G. Cheon:** None. **S. Lee:** None. **K. Kim:** None.

Poster

701. Peripheral Neuropathy

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 701.03/X13

Topic: E.10. Motor Neurons and Muscle

Support: GRF grant from the Research Grant Council of the Hong Kong Special Administrative Region Government (CityU 161212).

Title: Target expression of human heat shock protein 27 transgene in neurons promotes synapse reinnervations & functional recovery after prolonged muscle denervation

Authors: *P. ASTHANA[1], G. KUMAR[1], C. H. E. MA[1,2];
[1]Dept. of Biomed. Sci., City University of Hong Kong, Kowloon, Hong Kong; [2]Ctr. for Biosystems, Neuroscience, and Nanotechnology, City University of Hong Kong, Kowloon, Hong Kong

Abstract: Peripheral nerves are fragile and susceptible to physical trauma such as car accidents, traction injury, compression and lacerations. The slow growth rate of peripheral axons limits functional recovery in patients, which involve long-distance axonal regeneration. Delay in target muscle reinnervation makes the distal milieu non-permissive for the regenerating axons and the denervated muscle undergoes atrophy. Previous studies showed that these regenerating axons must arrive at the target muscle within a "critical period" of about 35 days in mice after multiple sciatic nerve crush (to delay muscle reinnervations), in order to restore motor function recovery. Our previous studies showed that forced expression of human Hsp27 (hHsp27) in neurons accelerated axonal regeneration and functional recovery in transgenic (Tg) mice after peripheral nerve injuries. In the present studies, sensory and motor functional recovery was significantly improved in hHsp27 Tg mice compared with the littermate (LM) controls. The number of axons in Tg and LM mice was comparable, however; higher number of fully innervated neuromuscular junction (NMJ) was observed in hHsp27 Tg mice than in the LM controls. Electrophysiological studies showed that muscle electrical activity increased considerably in hHsp27 Tg mice, which in line with the NMJ data. In addition, Plantar muscle mass was significantly higher in hHsp27 Tg mice than the LM controls, which showed reduction of muscle atrophy in Tg mice.

Disclosures: P. Asthana: 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; GRF grant from the Research Grant Council of the Hong Kong Special Administrative Region Government (CityU 161212).. **G. Kumar:** None. **C.H.E. Ma:** None.

Poster

701. Peripheral Neuropathy

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 701.04/X14

Topic: C.05. Neuromuscular Diseases

Support: Wellcome Trust Sir Henry Wellcome Postdoctoral Fellowship (103191/Z/13/Z)

French Muscular Dystrophy Association

Wellcome Trust Senior Investigator Award (107116/Z/15/Z)

University College London

UK Medical Research Council grant

NIH grant (NS054154)

Title: Sensory neuron fate is developmentally perturbed by Gars mutations in peripheral neuropathy

Authors: *J. N. SLEIGH[1], J. M. DAWES[2], S. J. WEST[2], E. L. SPAULDING[3], A. GÓMEZ-MARTÍN[1], R. W. BURGESS[3], M. Z. CADER[2], K. TALBOT[2], D. L. BENNETT[2], G. SCHIAVO[1];
[1]Sobell Dept. of Motor Neurosci. and Movement Disorders, Univ. Col. London, London, United Kingdom; [2]Nuffield Dept. of Clin. Neurosciences, Univ. of Oxford, Oxford, United Kingdom; [3]The Jackson Lab., Bar Harbor, ME

Abstract: Charcot-Marie-Tooth disease type 2D (CMT2D) is a peripheral nerve disorder caused by dominant, toxic, gain-of-function mutations in the widely expressed housekeeping gene, *GARS*. *GARS* encodes glycyl-tRNA synthetase (GlyRS), which serves to link glycine to its cognate transfer RNA, thereby ensuring the fidelity of the genetic code. The mechanisms underlying selective nerve pathology in CMT remain unresolved, as does the cause of the mild-to-moderate sensory involvement that distinguishes CMT2D from the allelic disorder distal spinal muscular atrophy type V. To elucidate the origin of afferent nerve pathology, we examined the sensory nervous system in CMT2D mice. We show that the equilibrium between functional subtypes of sensory neurons in dorsal root ganglia is distorted by *Gars* mutations, leading to sensory defects in peripheral tissues and correlating with overall disease severity. CMT2D mice display sensory behaviour deficits concordant with the afferent imbalance, which is present at birth and non-progressive. These data indicate that sensory neuron identity is prenatally perturbed in *Gars* mice, suggesting that both neurodevelopmental and neurodegenerative mechanisms contribute to CMT2D pathogenesis.

Disclosures: **J.N. Sleigh:** None. **J.M. Dawes:** None. **S.J. West:** None. **E.L. Spaulding:** None. **A. Gómez-Martín:** None. **R.W. Burgess:** None. **M.Z. Cader:** None. **K. Talbot:** None. **D.L. Bennett:** None. **G. Schiavo:** None.

Poster

701. Peripheral Neuropathy

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 701.05/DP03 (Dynamic Poster)

Topic: C.05. Neuromuscular Diseases

Support: NIH Grant K01 FOA PA-14-044

American College of Veterinary Surgeons Grant

University of Wiscosin School of Veterinary Medicine Companion Animal Grant

Title: Genetic basis of a naturally occurring canine model of peripheral neuropathy

Authors: *S. SAMPLE[1], P. MUIR[2], Z. HAO[2], H. RYLANDER[3], S. EMINAGA[3], H. BARNES HELLER[3], L. BAKER[2], E. BINVERSIE[2], A. PIAZZA[2], N. VOLSTAD[2], E. HANS[2], R. HARDIE[2], S. MURPHY[4], J. SVAREN[1];
[1]Dept. of Comparative Biosci., Univ. of Wisconsin Sch. of Vet. Medic, Madison, WI; [2]Dept. of Surgical Sci., [3]Dept. of Med. Sci., Univ. of Wisconsin-Madison, Madison, WI; [4]WestVet Animal Hosp., Garden City, ID

Abstract: Introduction: Inherited peripheral neuropathies are an important health concern for which there is currently no disease-modifying therapy. Dogs are affected by a variety of peripheral neuropathies that are breed-specific, indicating a strong genetic component. The use of in-bred populations, such as pure-bred dogs, is advantageous for genetic dissection of disease. Acquired peripheral neuropathy (APN) is an inherited late-onset generalized polyneuropathy with high prevalence in Labrador Retrievers. The most prominent features of APN, laryngeal paralysis and pelvic limb weakness, are associated with the longest peripheral motor nerves in the dog. The pathologic features of APN are similar to human peripheral neuropathy. The genetic basis of APN has not been determined.
Objective/Hypothesis: Our objective was to undertake a discovery genome-wide association study (GWAS) of APN in Labrador Retriever. APN is most likely a monogenic trait although more than one causal variant may explain a common phenotype.
Methods: We conducted a GWAS using 91 pure-bred Labrador Retrievers (n=65 cases, n=26 controls) using linear mixed model analysis with GEMMA. Direct siblings were excluded.
Case criteria: Dogs either received airway surgery for APN or had clinical signs of inspiratory noise and pelvic limb deficits indicating polyneuropathy. Dogs were excluded if evaluation suggested APN was not idiopathic.
Control criteria. Dogs usually exhibit signs by 11 years of age. Control dogs were ≥11.5 years of age with no clinical signs of respiratory obstruction and no evidence of polyneuropathy on neurologic examination. Dogs are continually monitored to confirm phenotype-negative status.

We used 118,952 SNP markers for analysis. Genome-wide significance was $P<2.7E-6$, based on Bonferroni correction of $P<0.05$ for 18,481 haplotype blocks using PLINK.
Results: This GWAS yielded a significant SNP association with the APN trait at $P=6.74E-7$. Genotypes-phenotype association analysis suggest that APN is an autosomal dominant Mendelian trait.
Discussion: We have identified a candidate locus in the dog genome that associates with a naturally occurring degenerative peripheral neuropathy with high prevalence in the most common dog breed in the USA. Our results suggest that APN is an autosomal dominant Mendelian trait in the Labrador Retriever. The work presented here is expected to enable discovery of the APN causal genetic variant. This work is a critical step towards development of a naturally occurring animal model for human peripheral neuropathy.

Disclosures: S. Sample: None. **P. Muir:** None. **Z. Hao:** None. **H. Rylander:** None. **S. Eminaga:** None. **H. Barnes Heller:** None. **L. Baker:** None. **E. Binversie:** None. **A. Piazza:** None. **N. Volstad:** None. **E. Hans:** None. **R. Hardie:** None. **S. Murphy:** None. **J. Svaren:** None.

Poster

701. Peripheral Neuropathy

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 701.06/X15

Topic: C.05. Neuromuscular Diseases

Support: Hereditary Neuropathy Foundation Grant

Title: Regulation of human peripheral myelin protein 22 by miR-29a

Authors: *L. NOTTERPEK[1], J. SERFECZ[2], A. FAN[2], S. LEE[2], R. RENNE[2];
[1]Neurosci., McKnight Brain Inst, Univ. Florida, Gainesville, FL; [2]Univ. of Florida, Gainesville, FL

Abstract: Peripheral myelin protein 22 (PMP22) is a dosage sensitive, disease-associated protein primarily expressed in myelinating Schwann cells. The restricted expression of the PMP22 protein in comparison to its ubiquitously detected mRNA supports a role for post-transcriptional regulation. MicroRNAs (miRNA) are small regulatory molecules that function at a post-transcriptional level by targeting predominantly 3'UTRs of mRNAs. Previously we demonstrated that in rat and mouse Schwann cells PMP22 is post-transcriptionally regulated by miRNAs, as over-expression of miR-29a reduces steady-state PMP22 levels, while inhibition of endogenous miR-29a relieves the miRNA-mediated repression of the gene (Verrier et al., 2009).

In the current study we asked if the human PMP22 transcript could be similarly inhibited by miR-29a in samples from Charcot-Marie-Tooth 1A (CMT1A) patients and in a rodent model of the disease. By bioinformatics we determined that the human PMP22, including the duplicated gene in CMT1A patients, indeed contains the full-length 3'UTR, and the conserved seed sequence of the miR-29a binding site. Next, using a Luciferase reporter assay in HEK293 cells we demonstrated that transfection with a miR-29a mimic, but not with a negative mimic control, is associated with an approximately 20% reduction in PMP22-like reporter activity. In dermal fibroblasts from CMT1A patients, we detected an elevated expression of PMP22 mRNA, which was effectively repressed by approximately 28-50% after transfection with a miR-29a mimic. As a control of specificity, a scrambled mimic did not lead to a reduction in PMP22 message levels. To further validate this approach, we cultured Schwann cells from the CMT1A mouse model named C22, which express approximately 8-10 fold elevated levels of the human PMP22 mRNA, as compared to the endogenous mouse mRNA. In agreement with data from the experiments in human fibroblasts, transfection with a miR29a mimic, but not with control scrambled miR, led to a reduction in the human PMP22 mRNA in cultured Schwann cells from the C22 mouse. Significantly, the human miR-29a mimic did not affect the expression of the mouse transcript, which indicates specificity of this approach. Together these results support further studies with miR29a as a potential therapeutic strategy to modulate increased expression of PMP22 in Schwann cells from CMT1A patients.

Disclosures: L. Notterpek: None. **J. Serfecz:** None. **A. Fan:** None. **S. Lee:** None. **R. Renne:** None.

Poster

702. Neuroprotective Mechanisms: Stress, Protein Trafficking, and Protein Degradation

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 702.01/X16

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: CNPq-Brazil

Title: Blockade of adenosine A_{2A} receptors protects against corticosterone effects in hippocampal neuronal cell line

Authors: *M. P. KASTER[1], N. PLATT[2], A. B. H. HRYB[2], F. N. KAUFMANN[2], M. P. CUNHA[2], A. COSTA[2], A. S. RODRIGUES[2];
[2]Dept. of Biochem., [1]Univ. Federal De Santa Catarina, Florianópolis, Brazil

Abstract: Stressful events may elicit different levels of arousal responses across individuals, leading to a continuum range of adaptive to extreme and maladaptive responses that are ultimately involved in disease manifestation. Indeed, hyperactivation of the hypothalamic-pituitary-adrenal (HPA) axis is a common finding in major depression, leading to increased levels of glucocorticoids, which are known to cause oxidative stress imbalance and cellular death, particularly in the hippocampus, a key region implicated in mood regulation. We recently demonstrated that blockade of adenosine A_{2A} receptors (A2AR) is capable to prevent behavioral and synaptic dysfunction in animal models of depression (Kaster et al., PNAS, 2015; Machado et al., Molecular Neurobiology, 2016). Thus, A2AR manipulation emerges as and attractive neuroprotective strategy to counteract stress effects in brain circuits. The main goal of this study was to investigate the effect of A2AR blockade in a model of stress induced by corticosterone in HT-22 hippocampal cell line. Western blot analysis demonstrated that HT-22 cells express glucocorticoid receptors (GR) and A2AR. Treatment with corticosterone significantly reduced the cellular viability evaluated by MTT reduction (24h, 50-1000 μM, $p<0.05$) and LDH release (24h, 500-1000uM, $p< 0.05$). Corticosterone (50 uM for 24h) also increased cell death assessed by propidium iodide ($p< 0.05$). The immunocontent of the pro-apoptotic protein Bax was increased after 24h incubation with corticosterone 50 uM, with no changes in Bcl-2 levels. Also, incubation of HT-22 cells with DCFDA demonstrated increased production of reactive oxygen and nitrogen species in the first 60 min of exposition. The results were compared using one-way ANOVA followed by Duncan *post-hoc* test).Pre-incubation (24h before) and co-incubation of ZM-241385 (a A2AR antagonist, 0.5 uM) and corticosterone (50 uM, 24h) was capable to prevent the effects of corticosterone on cell viability. These results were also obtained with the GR antagonist RU486 (2.5 e 5 μM) and with the selective serotonin reuptake inhibitor fluoxetine (10 uM). The results suggest that blockade of A2AR was capable to prevent the reduction in cell viability induced by corticosterone in HT-22 cells, suggesting that A2AR might represent an important strategy to counteract the effects of stress.

Disclosures: **M.P. Kaster:** None. **N. Platt:** None. **A.B.H. Hryb:** None. **F.N. Kaufmann:** None. **M.P. Cunha:** None. **A. Costa:** None. **A.S. Rodrigues:** None.

Poster

702. Neuroprotective Mechanisms: Stress, Protein Trafficking, and Protein Degradation

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 702.02/X17

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: IHDCYH 126790

IHDCYH 136908

Title: Characterization of M2 pathway following steroid exposure in neonatal microglia

Authors: *I. LONDONO[1], W. C. PIERRE[2], S. CHEMTOB[3], G. A. LODYGENSKY[3]; [1]Ste-Justine Hosp. Res. Ctr., Montreal, QC, Canada; [2]Pediatrics, [3]Pediatrics and Pharmacol., CHU Sainte Justine, Univ. de Montréal, Montreal, QC, Canada

Abstract: Introduction: Microglia activation is the primary hallmark of white-matter injury in preterm. Microglia phenotype in vivo and in vitro, following stimulation, has been described as pro-inflammatory (M1) and anti-inflammatory (M2). Corticosteroids such as dexamethasone (Dex) and hydrocortisone (HC) are well known anti-inflammatory agents extensively used to treat or prevent chronic bronchopulmonary dysplasia in preterm. Dex was mainly associated with impaired neurodevelopmental outcome. Objective: Compare the effect of hydrocortisone and dexamethasone on pro-inflammatory (M1) and anti-inflammatory (M2) pathways in activated neonatal microglia. Methods: Primary mixed glial cell cultures were prepared from whole brain of P1 Sprague-Dawley rats. Primary microglia culture were isolated from mixed glial cell cultures after 14 days in vitro and plated in 6-well plates at a density of $5x10^5$ cells/ml in DMEM supplemented with 10% FBS. Forty-eight hours later, microglia were treated with DMEM, LPS (1µg/ml) ± Dex (0.5µg/ml) or HC (13.33µg/ml) (n=3 for each condition). After 24h, cells were harvested and RNA was collected to evaluate the expression of M1 (iNOS and Il-1β) and M2 (Il-10 and arginase 1) related genes. Statistical comparisons between groups were done using Kruskal-Wallis tests. Results are presented as fold change to DMEM-treated microglia ±SEM. Results: LPS caused an activation of primary microglial cell toward an M1 phenotype as seen with the increased expression of iNOS and IL-1β. Co-incubation of Dex with LPS prevented the induction of M1 genes and induced significant but small increase in IL-10 expression. HC prevented the LPS-induced M1 phenotype in microglia without any effect on M2. Conclusion: Corticosteroids inhibited LPS-induced M1 activation phenotype in microglia. Dex induced a slight increase in the expression of IL-10, a known anti-inflammatory cytokine. An extensive study with more animals is underway evaluating a wide array of markers in each pathway. It appears that steroids don't have a large impact on neonatal M2 pathway.

qPCR of rat brain extract

	LPS	LPS+DEX	LPS+HC	p-value
Arginase 1	0.87±0.09	0.87±0.06	1.00±0.14	0.1669
IL-10	1.30±0.13	2.70±0.54	1.80±0.21	**0.0006**
IL-1β	75.00±18.00	1.60±0.23	1.20±0.19	**0.0044**
iNOS	5782±2473	66.00±32.00	2.50±0.71	**0.0006**

Disclosures: **I. Londono:** None. **W.C. Pierre:** None. **S. Chemtob:** None. **G.A. Lodygensky:** None.

Poster

702. Neuroprotective Mechanisms: Stress, Protein Trafficking, and Protein Degradation

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 702.03/X18

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NSERC Canada 6721

Title: Components of an Hsp70 disaggregation/refolding machine co-localize at nuclear speckles following thermal stress in differentiated human neuronal cells

Authors: ***C. A. DEANE**, I. R. BROWN;
Ctr. for the Neurobio. of Stress, Dept. of Biol. Sci., Univ. of Toronto Scarborough, Toronto, ON, Canada

Abstract: Heat shock proteins (Hsps) are a set of highly conserved proteins involved in cellular repair and protective mechanisms. They counter protein misfolding and aggregation that are characteristic features of neurodegenerative diseases. Hsps act co-operatively in disaggregation/refolding machines that assemble at intracellular sites of protein misfolding and aggregation. Members of the Hsp40 family act as 'holdases' to detect and bind misfolded proteins, which are then transferred to members of the Hsp70 family that act as 'foldases' to refold proteins to biologically active states. Hsp105α is an important additional member of the mammalian disaggregation/refolding machine that acts as a 'disaggregase' to promote the dissociation of aggregated proteins. Components of a disaggregation/refolding machine co-localized at nuclear speckles following thermal stress in differentiated human SH-SY5Y neuronal cells, namely: Hsp70-1 (HSPA1A), Hsp40-1 (DNAJB1), Hsp40-4 (DNAJA1) and Hsp105α (HSPH1). Nuclear speckles are rich in mRNA splicing factors, and heat shock is known to disrupt mRNA splicing which must recover following stressful stimuli. Interestingly, constitutively expressed Hsc70 (HSPA8) also co-localized at nuclear speckles with elements of a disaggregation/refolding machine following heat shock. Hence, neurons have the potential to rapidly assemble a disaggregation/refolding machine after cellular stress using constitutively expressed Hsc70 without the time lag needed for synthesis of stress-inducible Hsp70. Constitutive Hsc70 is abundant in particular classes of neurons in the mammalian brain and has been proposed to play a role in pre-protecting neurons from cellular stress.

Disclosures: **C.A. Deane:** None. **I.R. Brown:** None.

Poster

702. Neuroprotective Mechanisms: Stress, Protein Trafficking, and Protein Degradation

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 702.04/Y1

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: CB239607 CONACYT

DGAPA

Title: Hipoglucemia coma induces overexpression of endoplasmic reticulum stress markers and caspase activation in parietal cortex and hippocampus: B-hydroxybutyrate as an alternative energy source

Authors: *M. FLORES, T. MONTIEL, C. TORRES-ESQUIVEL, L. MASSIEU;
Inst. de Fisiología Celular, Univ. Nacional Autónoma de México, Mexico, Mexico

Abstract: The brain consumes 20% of the total body energy and therefore it requires a continuous supply of glucose. Glucose is the main energy source in the brain and when blood levels drop below 54 mg/dl, some symptoms arise such as irritability, dizziness, blurred vision, confusion and tachycardia. If this condition is not corrected and blood glucose decays to less than 20 mg/dl, the hypoglycemic coma (isoelectricity) might occur leading to neuronal death. It has been observed that under conditions of prolonged fasting, other energy substrates, such as the ketone body, D-B-hydroxybutyrate (BHB) can be used as energy source, as it is converted to acetyl coenzyme A (acetyl-CoA). In previous studies we have observed that rats subjected to severe hypoglycemia and treated with D-BHB, show decreased cell death in the cerebral cortex. Similarly, cortical cultures exposed to glucose deprivation in the presence of D-BHB, preserve ATP levels and show increased survival, suggesting that D-BHB can be used as an alternative energy source in these conditions (Julio-Amilpas et al. 2015. J.Cereb. Blood Flow Metab 35:851-60). One of the most energy demanding cellular processes is protein synthesis and disruption of the endoplasmic reticulum (ER) homeostasis during energy failure, can block protein synthesis through the unfolded protein response (UPR). The UPR is triggered through the activation of three sensors able of detect the accumulation of misfolded proteins. One of them is the PERK pathway; under ER stress, GRP78 dissociates from PERK and triggers its dimerization and activation. PERK phosphorylates eIF2a, which inhibits global protein synthesis, alleviating ER stress. However, if ER stress persists, eIF2a promotes the translation of the mRNA of the transcription factor ATF4, which up-regulates chop gene expression. CHOP protein promotes the mitochondrial apoptosis pathway by the down regulation of Bcl-2. We have observed that rats subjected to the hypoglycemic coma, show an increase in the protein content of GRP78, ATF4 and CHOP, as well as an increase in eIF2a phosphorylation in the parietal cortex and the hippocampus. We also observed the proteolytic activation of caspase-7 and caspase-12. These

effects are attenuated when rats are treated with BHB. Results suggest that the hypoglycemic coma induces ER stress, which is possibly involved in apoptotic neuronal death and that attenuation of ER stress by D-BHB treatment might contribute to its protective action against neuronal death.

Disclosures: **M. Flores:** None. **T. Montiel:** None. **C. Torres-Esquivel:** None. **L. Massieu:** None.

Poster

702. Neuroprotective Mechanisms: Stress, Protein Trafficking, and Protein Degradation

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 702.05/Y2

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Grant-in Aid for Scientific Research (C) 15K00814 from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) in Japan

Title: Histone deacetylase inhibitor MS-275 protects Neuro2a cells from endoplasmic reticulum stress via mitochondrial enhancement

Authors: ***K. NAGAI**, H. SHIRAZAWA;
Dept. of Nutr., Koshien Univ., Takarazuka-Shi, Japan

Abstract: Histone deacetylase (Hdac) inhibitors are focused on the preventing function against neurodegenerative diseases. However, the specificity of Hdac inhibition and the detailed mechanisms on neuroprotection are remained unsolved. We previously observed that Hdac inhibition protected Neuro2a cells from endoplasmic reticulum induced cell death which is known to causes some neurodegenerative diseases, and increased intracellular mitochondria in Neuro2a cells. Thus, we hypothesized that Hdac inhibition protected the neuronal cells via mitochondrial enhancement. In this study, we analyzed effects of subtype specific Hdac inhibitors on the mitochondrial activity and protective functions against endoplasmic reticulum stress inducers, tunicamycin, thapsigargin, or amyloid β 1-40 in Neuro2a cells. Mitochondrial activities were analyzed by image-based cytometry analysis of the cells stained with mitochondrial membrane potential dependent fluorescent dye JC-1. Broad Hdac inhibitor sodium butyrate increased the number of the cells with high amount of mitochondria. Regarding subtype specific Hdac inhibitors, Hdac1 and 3 specific inhibitor MS-275 increased the number of the cells with high amount of mitochondria, but Hdac2 specific inhibitor apicidin and class IIA Hdac inhibitor MC1568 did not. The mitochondria increasing effect of MS-275 was inhibited by bromodomain inhibitor JQ1. It suggests that the effect is Hdac associated transcription was

involved in the mitochondrial enhancement. Regarding cell protective effect, MS-275 attenuated tunicamycin, thapsigargin, and amyloid β 1-40 toxicity. And these protective effects were reduced in the presence of JQ1. It suggests that inhibition of Hdac 1 and/or 3 protects Neuro2a cells from endoplasmic reticulum stress induced toxicity. Our data suggest that inhibition of Hdac 1 and/or 3 protects neuronal cells from endoplasmic reticulum stress via mitochondrial enhancement, and the mitochondrial effect might contribute neurodegeneration preventing effects of Hdac inhibitors.

Disclosures: K. Nagai: None. **H. Shirazawa:** None.

Poster

702. Neuroprotective Mechanisms: Stress, Protein Trafficking, and Protein Degradation

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 702.06/Y3

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Regione Autonoma della Sardegna L.R. n.7/2007-CRP10810/2012

Title: Inhibition of autophagy by activation of M_3 muscarinic acetylcholine receptors in human SH-SY5Y neuroblastoma cells.

Authors: *P. ONALI, S. DEDONI, M. C. OLIANAS;
Univ. of Cagliari, Dept.Biomedical Sci., Monserrato, Italy

Abstract: Muscarinic acetylcholine receptors (mAChRs) have been shown to regulate neuronal cell proliferation and differentiation and to protect neuronal cells from apoptosis induced by a variety of insults. However, relatively little is known on the ability of mAChRs to regulate autophagy, an evolutionary conserved cellular response to different physio-pathological conditions. In the present study, we investigated the effect of mAChR stimulation on autophagy induced in human SH-SY5Y neuroblastoma cells by removal of serum and L-glutamine from the growth medium. Autophagy was monitored by measuring the levels of the autophagosome marker LC3-II and the autophagy substrate p62. We found that cell exposure to the cholinergic agonist carbachol (CCh) (30 μM) inhibited LC3-II accumulation and enhanced the levels of p62. The effects of CCh were mimicked by the mAChR agonist oxotremorine-M (100 μM) and blocked by atropine or the M_3 mAChR-preferring antagonist darifenacin, but not MT7, a selective M_1 mAChR blocker. CCh inhibition of autophagy was counteracted by blockade of $G_{q/11}$ with YM254890 and protein kinase C (PKC) with Go6983, bisindolylmaleimide and chronic treatmen with phorbol 12-myristate 13-acetate. Cell treatment with the mammalian target of rapamycin complex 1 (mTORC1) antagonist rapamycin completely suppressed CCh-induced

phosphorylation of S6 ribosomal protein, a mTORC1 target, and prevented the inhibitory effect of CCh on autophagy. These data indicate that in SH-SY5Y cells activation of $G_{q/11}$-coupled M_3 mAChRs inhibits starvation-induced autophagy by activating the PKC-mTORC1 signaling pathway.

Disclosures: **P. Onali:** None. **S. Dedoni:** None. **M.C. Olianas:** None.

Poster

702. Neuroprotective Mechanisms: Stress, Protein Trafficking, and Protein Degradation

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 702.07/Y4

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Ceftriaxone attenuates chronic mild stress induced spatial learning and memory deficits. Correlation with hippocampal NeuN and c-Fos expression.

Authors: ***J. A. SCHROEDER**[1], L. PEREZ[1], L. MININBERG[1], S. PIERCE[1], E. L. GOMYAW[1], S. F. KORNACKI[1], G. UMAN[1], R. CAREDENAS[1], A. F. WARREN[1], S. M. RAWLS[2];
[1]Dept Psychol, Connecticut Coll, New London, CT; [2]Pharmacol., Temple Univ. Sch. of Med., Philadelphia, PA

Abstract: Prolonged exposure to stress can result in a depressive state that is associated with hippocampal damage and learning and memory deficits that may be related to increased brain glutamate levels. The beta-lactam antibiotic ceftriaxone reduces extracellular glutamate by activating the glutamate transporter and enhancing synaptic glutamate clearance. The present study examined the effects of ceftriaxone on chronic mid stress (CMS)-induced depressive-like behavior and spatial learning and memory performance in rats. Twenty-five day CMS did not result in depressive-like behavior measured by sucrose preference and open field tests. However CMS resulted in acquisition and expression learning and memory deficits in Morris Water Maze performance. These deficits were attenuated by administration of ceftriaxone during stress exposure. Ongoing immunohistochemical studies will attempt to correlate hippocampal expression of c-Fos and NeuN with behavioral measures. These results suggest that stress-induced deficits in learning and memory are associated with increased brain glutamate levels and that these deficits can be prevented by enhancement of synaptic glutamate clearance.

Disclosures: **J.A. Schroeder:** None. **L. Perez:** None. **L. Mininberg:** None. **S. Pierce:** None. **E.L. Gomyaw:** None. **S.F. Kornacki:** None. **G. Uman:** None. **R. Caredenas:** None. **A.F. Warren:** None. **S.M. Rawls:** None.

Poster

702. Neuroprotective Mechanisms: Stress, Protein Trafficking, and Protein Degradation

Location: Halls B-H

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Topic: C.06. 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 inositol 1,4,5-trisphosphate receptor mediated calcium signaling by steroid hormone receptors

Authors: *P. KOULEN, A. A. LOPEZ, J. C. MEANS;
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 inositol 1,4,5-trisphosphate 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 inositol 1,4,5-trisphosphate 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 inositol 1,4,5-trisphosphate 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 inositol 1,4,5-trisphosphate 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. **A.A. Lopez:** None. **J.C. Means:** None.

Poster

702. Neuroprotective Mechanisms: Stress, Protein Trafficking, and Protein Degradation

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 702.09/Y6

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Research Sponsored by XoNovo

Title: XN-001, a novel drug for neurodegeneration via CRMP2 activating pathways

Authors: ***K. HENSLEY**[1], K. VENKOVA-HRISTOVA[2], A. HRISTOV[2], M. HARRIS-WHITE[3], C. HORST-LILLIG[4], M. GELLERT[4], P. KURSULA[5], L. BRAIMAN-WIKSMAN[6], I. NEVO[6];
[2]Pathology, [1]Univ. of Toledo Med. Ctr., Toledo, OH; [3]Greater Los Angeles Veteran's Affairs Healthcare Syst., Los Angeles, CA; [4]Inst. fur Medizinische Biochemie und Molekularbiologie, Griefswald Univ., Griefswald, Germany; [5]Dept. of Biomedicine, Univ. of Bergen, Bergen, Norway; [6]XoNovo Ltd., Ness Ziona, Israel

Abstract: Because different neurodegenerative diseases share common pathological elements including neuroinflammation, calcium dyshomeostasis, excitotoxicity, blocked autophagy and cytoskeletal disruption it seems plausible that common junction points in cellular pathophysiology might be found such that one could treat a broad spectrum of neurodegenerative disorders with the same therapeutic agent. We have accumulated a body of data that bioavailable derivatives of the natural brain sulfur amino acid metabolite, lanthionine ketimine (LK) have potent neuroprotective and neurotrophic properties and treat preclinical models of diverse neurodegenerative conditions by a novel mechanism. In particular LK-ethyl ester (XN001) slows disease or reduces histopathological correlates of injury in mouse models of amyotrophic lateral sclerosis (ALS); Alzheimer's disease; Batten disease (BD); glioma; multiple sclerosis; stroke and traumatic brain injury. XN001 acts in a novel fashion to bind the microtubule-associated protein CRMP2 (collapsin response mediator protein-2) to influence its conformation and sensitivity to phosphorylation. In stem cell derived neurons XN001 inhibits CRMP2 phosphorylation and promotes complex association of CRMP2 with Vps34 autophagy complex including AMBRA1 and beclin-1 and induces autophagy flux. In iPSC-derived neurons from Batten disease patients bearing a common 1kb deletion in *Cln3* (Battenin), CRMP2 phosphorylation is increased and autophagy flux is decreased which underlies CLN3-linked BD pathology. XN001 overcomes these impairments and normalizes CRMP2 phosphorylation as well as autophagy progression. In the $Cln3^{\Delta ex7/8}$ mouse model of BD, we show that in aging $Cln3^{\Delta ex7/8}$ mouse brain CRMP2

becomes hyperphosphorylated. Of practical importance to BD, XN001 significantly reduces autofluorescent ceroid in the cerebellum of these mice. These findings are discussed with reference to possible clinical utility of XN001 to treat a broad spectrum of neurodegenerative diseases involving pathological hyperactivation of CRMP2. KH is inventor of LKE; KH, LBW, and IN have financial interests in XoNovo.

Disclosures: **K. Hensley:** 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; XoNovo Ltd.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); XoNovo Ltd. **K. Venkova-Hristova:** None. **A. Hristov:** None. **M. Harris-White:** None. **C. Horst-Lillig:** None. **M. Gellert:** None. **P. Kursula:** None. **L. Braiman-Wiksman:** A. Employment/Salary (full or part-time): XoNovo. **I. Nevo:** A. Employment/Salary (full or part-time): XoNovo Ltd..

Poster

702. Neuroprotective Mechanisms: Stress, Protein Trafficking, and Protein Degradation

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 702.10/Y7

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NINDS Grant 1R15NS093594-01A1

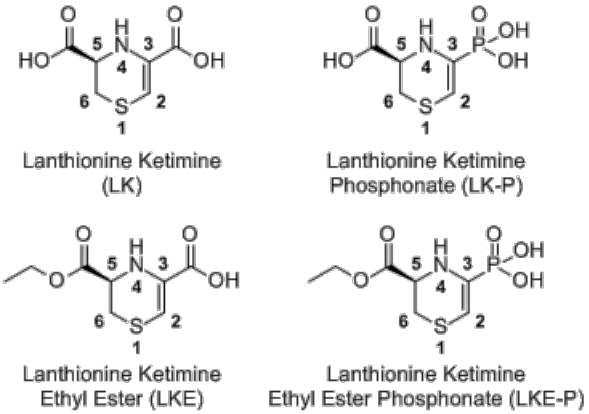
WSU Startup Funds

Title: Phosphonate analogues of lanthionine ketimine as stimulators of cellular autophagy: development of small molecule treatments for neurological disorders

Authors: ***T. T. DENTON**[1], D. SHEN[2], A. HRISTOV[3], K. VENKOVA-HRISTOVA[3], K. HENSLEY[3];
[1]Washington State Univ. Col. of Pharm., Spokane, WA; [2]Pharmaceut. Sci., Washington State Univ. Col. of Pharm., Spoknae, WA; [3]Pathology, Univ. of Toledo, Toledo, OH

Abstract: Lanthionine ketimine (LK) is a natural amino acid metabolite found at low concentrations in mammalian brain tissue. LK and its synthetic ester derivative, LKE (XN-001) have potent neuroprotective, neurotrophic and anti-neuroinflammatory properties and increase autophagy in neurons and glia. In published and ongoing studies, LKE shows benefit in diverse preclinical models of Alzheimer's disease; amyotrophic lateral sclerosis; Batten disease; glioma; multiple sclerosis; traumatic brain injury and stroke. Using LK and LKE as lead compounds, we

have recently developed novel chemical approaches that allow access to new regions of structure-activity space around the LK core structure by replacing the carboxyl group on position 3 of LK with a phosphonate group (LK-P) either with retention or removal of the carboxylate group on position 5. We are assessing bioavailability and activity in an RG2 glioma cell based autophagy assay, employing quantitative western blot densitometry, wherein the new compounds were shown to increase autophagic flux evidenced by the compounds' ability to increase phosphatidylethanolamine conjugation to microtubule associated protein 1 light chain 3 (LC3-I ➔LC3-II conversion) in the presence of bafilomycin-A1. These results suggest that phosphonate analogues of LK retain cell penetrating ability and bioactivity with respect to autophagy stimulation in glial cell culture.



Disclosures: **T.T. Denton:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); TD has intellectual property related to data in the presentation. **D. Shen:** None. **A. Hristov:** None. **K. Venkova-Hristova:** None. **K. Hensley:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); KH has intellectual property related to data in the presentation and KH has equity in a company developing LK derivatives for neurological disease.

Poster

703. Non-Pharmacological and Molecular Strategies to Prevent Injury and Stroke.

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 703.01/Y8

Topic: C.09. Brain Injury and Trauma

Support: Startup Research Funds, New York Medical College

Title: Acute and chronic tissue effects of focused ultrasound neuromodulation assessed at the cellular and bulk tissue scale

Authors: *I. GUMENCHUK, J. A. N. FISHER;
Physiol., New York Med. Col., Valhalla, NY

Abstract: The use of transcranial low intensity focused ultrasound (FUS) is an emerging neuromodulation technique that shows promise for both therapeutic and research applications. In humans, for example, FUS can modulate EEG (Legon et al., *Nat. Neurosci.* 2014) and has been shown to affect mood (Hameroff et al., *Brain Stim.* 2013), suggesting general utility in treating psychiatric conditions for which the neural basis is not well understood. Compared with other noninvasive neuromodulation techniques such as transcranial magnetic stimulation (TMS), key technical advantages include high lateral resolution of stimulation (millimeter scale) at deep penetration depths (~10 cm) as well as increased MRI compatibility. With an eye toward eventual clinical implementation of the technology, a central criterion of regulatory agencies is that radiation emitting devices do not cause tissue modification. We explored potential biomarkers for adverse tissue effects by using real-time optical imaging as well as histological analysis following exposure to FUS with parameters previously utilized in the literature. Using a custom ultrasonic transducer apparatus that permits simultaneous optical imaging at the focus, we observed in brain slices, above ultrasound intensities sufficient to evoke motor responses but well below the regime of high-intensity focused ultrasound (HIFU), the emergence of focal lesions with increasing durations of exposure to pulsed ultrasound. The mechanical effect varied depending on the targeted brain region and stimulation parameters, and differed as well between the mouse and rat. At longer FUS exposures, we observed in survival experiments elevated levels of glial fibrillary acidic protein (GFAP) expression as well as evidence of neurodegeneration (via Fluoro Jade staining) as early as 24 hours post FUS and persisting at least 72 hours. At shorter US exposures, we observed less intense, diffuse patterns of expression and neurodegeneration that were nonetheless confined to the hemisphere of sonication. Consistent with the measured axial intensity profile at the acoustic focus, altered GFAP and Fluoro Jade labeling was observed at regions well below the cortex, most notably in the hippocampus and corpus callosum. Our results suggest that rather than a specific damage threshold, there appears to be a gradation of chronic tissue effects that result from exposure to the upper range of previously employed FUS neuromodulation parameters.

Disclosures: I. Gumenchuk: None. **J.A.N. Fisher:** None.

Poster

703. Non-Pharmacological and Molecular Strategies to Prevent Injury and Stroke.

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 703.02/Y9

Topic: C.09. Brain Injury and Trauma

Title: The treatment of chronic craniofacial pain with novel gene therapy approaches.

Authors: *O. P. KEIFER, JR[1], J. MADER[2], N. M. BOULIS[2];
[2]Dept. of Neurosurg., [1]Emory Univ. Sch. of Med., Atlanta, GA

Abstract: Chronic craniofacial pain is a devastating condition that significantly disables those who suffer from it. Despite being a well-known disease entity, little is understood about the pathophysiology. Further, there are few treatment options that provide any consistent or long-term solutions to the chronic pain (treatments may include pharmacological approaches or surgical approaches). Thus, the treatment of patients with chronic craniofacial pain presents a challenge to clinicians and researchers. In a readdress, we are presenting novel application of gene-based neuromodulation for the control of craniofacial pain, using established rodent models of Temporomandibular joint disorder (TMJD) and Trigeminal Inflammatory Compression (TIC) to assess efficacy. The TMJD model is a model for chronic nociceptive pain (pain caused by stimulation of pain receptors), while the TIC model is a model for neuropathic pain (pain caused by injury to the nerve). We show proof-of-concept results for the delivery of transgenes that are designed to affect the process of neural transmission and were delivered to the trigeminal pain pathway (i.e. pathways such as the Trigeminal Ganglion and Spinal Nucleus of V). The utility of different viral vector-based delivery systems and routes is also discussed for the two rodent models and compared. As a translational laboratory, our ultimate intention is to develop a gene-based neuromodulatory therapy that can be validated through human clinical trials.

Disclosures: O.P. Keifer: None. **J. Mader:** None. **N.M. Boulis:** None.

Poster

703. Non-Pharmacological and Molecular Strategies to Prevent Injury and Stroke.

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 703.03/Y10

Topic: C.08.Stroke

Support: NIH grant NS085272

Title: Biochanin A protects against stroke by induction of glutamate oxaloacetate transaminase

Authors: *S. KHANNA, R. STEWART, S. GNYAWALI, S. ROY, C. K. SEN, C. L. RINK; Surgery, Ohio State Univ., Columbus, OH

Abstract: Glutamate serves multi-faceted (patho)physiological functions in the central nervous system as the most abundant excitatory neurotransmitter and under pathological conditions of ischemic stroke as a potent neurotoxin. Glutamate oxaloacetate transaminase (GOT) has emerged as a new therapeutic target against ischemic stroke by metabolizing neurotoxic glutamate. In search of a small molecule inducer of GOT, we identified Biochanin A (BCA) as the most potent phytoestrogen isoflavone that increases GOT expression in neuronal cells. Here we hypothesize that BCA induces GOT expression by an estrogen related receptor alpha (ERRα) dependent mechanism. To test this hypothesis, neuronal cells in culture were treated with BCA. BCA treated cells showed significant increase in ERRα mRNA at 6h and GOT mRNA and protein expression at 24h. Cells treated with BCA were significantly protected against glutamate toxicity. Notably, this protection was lost in BCA treated cells when GOT was knocked down. To validate these findings in vivo, C57Bl/6 mice were intraperitoneally injected with either vehicle control (75% DMSO in water) or BCA (5 or 10 mg/kg body weight) daily for 4 weeks. Following delivery, mice were subjected to ischemic stroke using the intraluminal thread method of middle cerebral artery occlusion (MCAO). BCA levels were significantly increased in blood and brain of IP-injected mice as measured by HPLC. Immunohistochemical analysis confirmed increased expression of GOT in brain. BCA treatment significantly improved post-stroke sensorimotor function and attenuated stroke-induced lesion volume as measured by MRI. In this work we have identified BCA as a natural small molecule inducer of GOT. BCA is a safe, naturally occurring phytoestrogen isoflavone that represents a therapeutic target that lends itself to be translated to clinical study.

Disclosures: **S. Khanna:** None. **R. Stewart:** None. **S. Gnyawali:** None. **S. Roy:** None. **C.K. Sen:** None. **C.L. Rink:** None.

Poster

703. Non-Pharmacological and Molecular Strategies to Prevent Injury and Stroke.

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 703.04/Y11

Topic: C.08.Stroke

Support: NIH R01NS046400

NIH F31NS086441

McKnight Brain Institute

Title: CD163 has distinct temporal influences on intracerebral hemorrhage outcomes

Authors: *J. L. LECLERC, A. LAMPERT, C. LOYOLA AMADOR, B. SCHLACKMAN, S. DORE;
Anesthesiol., Univ. of Florida, Gainesville, FL

Abstract: Hemoglobin (Hb)/heme-induced toxicity following intracerebral hemorrhage (ICH) is a precipitating factor for neuroinflammation and secondary brain injury, which results in irreversible brain damage and enduring neurological deficits. The haptoglobin (Hp)-CD163 scavenging system is the primary defense mechanism in the body against the deleterious effects of extracorpuscular Hb/heme. Hp immediately and irreversibly binds any free Hb, and the complex is endocytosed by cells of the monocyte lineage via the CD163 receptor. When Hp levels are depleted, CD163 has been reported to directly decrease the toxic effects of Hb through direct binding. CD163 also has potent anti-inflammatory properties participating in the downregulation of inflammatory responses. This study is the first to investigate the role of CD163 after ICH. ICH was induced in wildtype and CD163-/- mice and various anatomical and functional outcomes were temporally assessed by histology and neurobehavioral testing. At 72h, CD163-/- mice have 33.2±4.5% and 43.4±5.0% smaller lesion and hematoma volumes, respectively, which are associated with 34.8±3.4% less perihematomal tissue injury (n=19-20/group, $p<0.0001$). These anatomical improvements were accompanied by significantly less neurological deficits, as identified by neurological deficit scoring and rotarod performance. At 10d, CD163-/- mice have 49.2±15.0% larger lesion volumes and worse neurological deficits, as identified by neurological deficit scoring and open field ambulatory ability (n=11-12/group, $p<0.05$). Mortality analyses and regressions of the neurobehavioral results identified the inflection point at 4d post-ICH. Mortality on or before day 4 (total deaths, n=13) and after day 4 (total deaths, n=9) significantly differed between the groups ($p = 0.0390$). WT mice accounted for the majority of deaths on or before day 4 compared to CD163 mice (76.9% vs. 23.1%). Immunohistochemical staining for heme oxygenase 1, iron, GFAP, IgG, Hb, VEGF, and PECAM-1 also revealed distinct temporal-dependent mechanistic changes. These novel findings reveal that CD163 has distinct temporal influences on ICH outcomes, with early beneficial effects but delayed injurious properties, signifying a discrete therapeutic window.

Disclosures: J.L. Leclerc: None. **A. Lampert:** None. **C. Loyola Amador:** None. **B. Schlackman:** None. **S. Dore:** None.

Poster

703. Non-Pharmacological and Molecular Strategies to Prevent Injury and Stroke.

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 703.05/Y12

Topic: C.09. Brain Injury and Trauma

Support: NIH-Waisman IDDRC P30HD003352

NIH K08 NS088563

UL1TR0000427

KL2TR000428

Department of Pediatrics R and D Grant

Title: Sexually differentiated TrkB phosphorylation is dependent on estrogen receptor alpha in hippocampal neurons

Authors: D. ZAFER[1], V. CHANANA[1], A. CANTURK[1], D. KINTNER[1], J. H. CHANDRASHEKTAR[1], E. UDHO[1], P. FERRAZZANO[1], R. A. SHAPIRO[1], J. E. LEVINE[1], *P. CENGIZ[2];
[1]UW-Madison, Madison, WI; [2]Dept. Pediatrics and Waisman Center, Univ. of Wisconsin-Madison, Madison, WI

Abstract: Male neonate brains are more susceptible to the effects of hypoxia-ischemia (HI) related brain injury. Sex differences in expression and actions of neurotrophins may account for sexually differentiated consequences of HI. Our recent findings reveal that tyrosine kinase B receptor (TrkB) agonist, 7,8-dihydroxyflavone (7,8-DHF), exerts a profound neuroprotective effect in the hippocampi of female but not male neonate mice through phosphorylation of the TrkB post-HI *(in-vivo)*. Differential hippocampal TrkB phosphorylation is associated with increased hippocampal ERα expression in $ER\alpha^{+/+}$ female mice and gets ablated in $ER\alpha^{-/-}$ female mice. These results suggest a role of ERα in conferring responsivess to TrkB phosphorylation in female mice only. We hypothesized that differential ERα expression followed by TrkB phosphorylation and neuroprotection takes place in hippocampal neurons after in-vitro ischemia. Sexed hippocampal primary neuronal cultures were prepared from P1 C57BL/6J $ER\alpha^{+/+}$ and $ER\alpha^{-/-}$ mice in estrogen free medium and exposed to either normoxia or OGD for 4 h at DIV 7 followed by VC or 7,8-DHF treatment. After 24 h REOX, cells were stained for cell survival and p-TrkB. Expressions of ERα and aromatase were performed 3 and 24 of REOX using qPCR. For multiple comparisons ANOVA was used. 7,8-DHF enhanced TrkB phosphorylation in a dose responsive manner and promoted cell survival only in $ER\alpha^{+/+}$ female hippocampal neurons following OGD-REOX ($p < 0.05$). HI and 7,8-DHF mediated increases in TrkB phosphorylation

was ablated in ERα$^{-/-}$ male and female hippocampal neurons. Four h of OGD and 3 h of REOX induced higher ERα mRNA expression only in ERα$^{+/+}$ female hippocampal neurons. No differences were detected in aromatase mRNA expressions. Sexually differentiated TrkB phosphorylation in response to *in-vitro* ischemia enhanced with TrkB agonist therapy in the female hippocampal neurons is dependent on ERα and can be ligand independent. Future research will attempt to identify the mechanisms in which TrkB and ERα are related following *in-vitro* ischemia in hippocampal neurons. By understanding the sexually differential role of ERα-TrkB interaction in neuronal survival, we hope to provide novel insights into the etiology and targeted therapies post- HI.

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Poster

703. Non-Pharmacological and Molecular Strategies to Prevent Injury and Stroke.

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 703.06/Y13

Topic: C.08.Stroke

Title: *In vitro* inhibition of tissue plasminogen activator induced neutrophil degranulation is time dependent following acute brain injury

Authors: *I. IWUCHUKWU[1], D. NGUYEN[2], N. MAHALE[2], L. ALLULLI[2], O. SULAIMAN[3];
[1]NEUROLOGY, Ochsner Hlth. Syst., New Orleans, LA; [2]Inst. of Translational Med., [3]Neurosurg., Ochsner Med. Ctr., New Orleans, LA

Abstract: Background Neutrophils are rapidly mobilized following acute brain injury and associated with MMP-9 release due to degranulation. Elevated MMP-9 levels are associated with blood brain barrier damage, cerebral edema, hemorrhagic transformation and expansion. TPA induces neutrophil degranulation. In this study, we compared the inhibition of TPA-induced neutrophil degranulation and MMP-9 release ex vivo, in neutrophils isolated from healthy individuals and patients with intracerebral hemorrhage (ICH). Methods Neutrophils were isolated from blood samples obtained from patients with ICH (within 24 hrs of symptom onset and daily; admission days 1-4) and healthy controls (N=4). Isolated neutrophils were plated on a 24-well poly-L-lysine coated tissue dish. This was followed by preincubation with sulfasalazine or pentoxyfylline for 30mins followed by addition of TPA (3mg/ml) and then incubated for 20, 30, 40 and 60mins. Cell media was removed and analyzed for proMMP-9 activity by gelatin gel

zymography. Band intensity corresponding to pro-MMP9 and active MMP-9 were quantified using ChemiDoc MP Imager. Results In both healthy controls and ICH patients, degranulation and release of proMMP-9 was observed in neutrophils incubated with TPA at all time points. In healthy controls, preincubation with sulfasalazine (10uM, 20uM, 50uM and 100uM) or pentoxyfyline (1nM) for 30mins followed by addition of TPA achieved 50% reduction in release of proMMP9 at all time points. However, in neutrophils obtained from ICH patients, 50% reduction in proMMP-9 was only observed in neutrophils obtained within 24 hrs of symptom onset (admission day 1 of ICH). Sulfasalazine and pentoxyfyline had no effect in blocking TPA induced degranulation of neutrophils obtained greater than 24 hrs of symptom onset (admission day 2-4 of ICH). Conclusion Sulfasalazine and pentoxyfyline ex-vivo inhibition of TPA induced neutrophil degranulation is more effective in the early phase of acute brain injury (within 24 hrs of symptom onset) but not in later isolations likely as a result of neutrophil priming and activation.

Disclosures: I. Iwuchukwu: None. **D. Nguyen:** None. **N. Mahale:** None. **L. Allulli:** None. **O. Sulaiman:** None.

Poster

703. Non-Pharmacological and Molecular Strategies to Prevent Injury and Stroke.

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 703.07/Y14

Topic: C.09. Brain Injury and Trauma

Support: NIH Grant AG044404

Title: TNFα alters occludin and cerebral endothelial permeability: role of p38MAPK

Authors: *G. Y. SUN[1], Y. NI[1], T. TENG[2], J. LEE[2];
[1]Univ. Missouri, Columbia, MO; [2]Univ. of Illinois at Chicago, Chicago, IL

Abstract: Occludin is one of the key tight junction (TJ) proteins in endothelial cells and it plays an important role in modulating blood brain barrier (BBB) function. This protein (65kDa) has been shown to engage in many signaling pathways and subjected to phosphorylation by a number of protein kinases. Activation of endothelial cells by pro-inflammatory cytokines and endotoxin (lipopolysaccharides, LPS) may alter TJ proteins and BBB functions. Here we describe the responses of occludin in immortalized human cerebral endothelial cells (hCMEC/D3 cells) stimulated by TNFα, IL-1β and LPS. Exposing cells to TNFα resulted in a rapid and transient band shift of occludin suggesting an increase in phosphorylation whereas IL-1β and LPS produced significantly less effects on the band shift. TNFα also caused transient stimulation

of p38MAPK and ERK1/2 in hCMEC/D3 cells, and TNFα-induced occludin phosphorylation was suppressed by SB202190, inhibitor for p38MAPK. Cells treated with TNFα for 24h resulted in cell morphology changes, a decrease in the expression of occludin, and enhanced endothelial permeability, as determined by the FITC-dextran assay and TEER measurement with cells grown in transwell inserts. In addition, TNFα-induced reduction of occludin was abrogated by SB202190. Collectively, these data demonstrate effects of TNFα on occludin and cerebral endothelial cell function through the activation of p38MAPK pathway.

Disclosures: G.Y. Sun: None. **Y. Ni:** None. **T. Teng:** None. **J. Lee:** None.

Poster

703. Non-Pharmacological and Molecular Strategies to Prevent Injury and Stroke.

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Program#/Poster#: 703.08/Y15

Topic: C.09. Brain Injury and Trauma

Support: International Foundation for Research in Paraplegia

Wings for Life Spinal Cord Research Foundation

Swiss National Science Foundation

NIH grant EY021242

NIH grant P30 HD018655

NIH grant P30EY012196

Title: The mammalian specific protein ARMCX1 promotes mitochondrial transport, neuronal survival and axonal regeneration

Authors: *R. CARTONI[1], M. W. NORSWORTHY[1], F. BEI[1], C. WANG[1], S. LI[1], C. V. GABEL[2], T. L. SCHWARZ[1], Z. HE[1];
[1]Neurol., Boston Children's Hospital/Harvard Med. Sch., Boston, MA; [2]Physiol. and biophysics, Photonic Ctr., Boston Univ., Boston, MA

Abstract: Whether and how mitochondrial dynamics impact neuronal injury responses, such as neuronal survival and axon regeneration, remain unknown and untested. Here we find that ARMCX1, a mammalian-specific gene that encodes a mitochondrial localized protein, was significantly up regulated in injured mouse retinal ganglion cells (RGCs) with co-deletion of PTEN and SOCS3 (dKO), an established model of robust axon regeneration. ARMCX1 over-

expression enhances mitochondrial transport in both adult RGCs and embryonic mouse cortical neurons. ARMCX1 is necessary for dKO RGCs axonal regeneration and neuronal survival as ARMCX1 knockdown drastically impaired axonal re-growth and neuroprotection after optic nerve crush in adult mice. ARMCX1, but not an ARMCX1 mutant that does not localize to mitochondria, promotes both neuronal survival and axonal regeneration. Moreover, ARCMX1 can induce axon regenerations from RGC subtypes that are refractory to PTEN-deletion manipulation alone. Thus ARMCX1 represents a potential new target for designing neural protective and repair strategies after injury.

Disclosures: R. Cartoni: None. **M.W. Norsworthy:** None. **F. Bei:** None. **C. Wang:** None. **S. Li:** None. **C.V. Gabel:** None. **T.L. Schwarz:** None. **Z. He:** None.

Poster

703. Non-Pharmacological and Molecular Strategies to Prevent Injury and Stroke.

Location: Halls B-H

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Program#/Poster#: 703.09/Y16

Topic: C.09. Brain Injury and Trauma

Support: CURE

F.M. Kirby Foundations

NJCBIR CBIR14RG024 to VS

NJCBIR CBIR15FEL011 to AK

Title: Distinct signaling pathways underlie neurophysiological effects of toll-like receptor 4 signaling in the normal and injured brain

Authors: *A. A. KORGAONKAR[1], J. GUEVARRA[1], K. C. H. PANG[1,2], V. SANTHAKUMAR[1];
[1]Dept. of Pharmacology, Physiol. and Neurosciences, Rutgers New Jersey Med. Sch., Newark, NJ; [2]Neurobehavioral Res. Lab., Veteran Affairs Med. Center–New Jersey Hlth. Care Syst., East Orange, NJ

Abstract: Concussive brain injury results in neuronal degeneration, reactive astrocytosis and enhanced excitability in the hippocampal dentate gyrus, increasing the risk for epilepsy and memory dysfunction. Endogenous molecules released during injury can activate innate immune responses including toll-like receptor 4 (TLR4). Enhanced neuronal TLR4 signaling contributes to an increase in dentate excitability after TBI. In contrast, TLR4 agonists reduced excitability in

uninjured rats suggesting that TLR4 signaling engages distinct molecular effectors in the control and injured brain. Here, we examine potential mechanisms and consequences of suppressing TLR4 signaling. Wistar rats (25-27 days old) were subject to sham- or moderate fluid percussion injury (FPI). The effects of specific antagonists on perforant path-evoked dentate population responses were analyzed in hippocampal slices obtained one week after injury. Neurophysiological outcomes were examined in rats treated with focal (LPS-RS-U) or systemic (CLI-095) TLR4 antagonist or vehicle. Similar to acute incubation, CLI-095 (i.p) decreased afferent-evoked dentate population spike amplitude 1 week after FPI yet enhanced dentate excitability in shams. Pre-incubation of hippocampal slices in glial metabolic inhibitors blocked TLR4 ligand modulation of dentate excitability in shams but not after FPI. TNFα inhibitor eliminated TLR4 agonist modulation of excitability in slices from both sham and FPI suggesting a novel neuronal TLR4-TNFα signaling after brain injury. Working memory function on a delayed match to sample Morris Water Maze task was impaired for one month after FPI and 62% of FPI rats developed of spontaneous seizures. Both focal and systemic TLR4 antagonist administration 24 hrs after FPI improved working memory function at 1 week and 1 month post-FPI. The treatments also prolonged the latency to kainic acid induced seizures and reduced seizure severity (by Racine scale) after injury. The same treatments impaired working memory function and enhanced seizure severity in shams. Delayed TLR4 antagonism one month after injury failed to limit seizure susceptibility. These data demonstrate differential involvement of glia and identify a potential role of neuronal TNFα in TLR4 signaling in the normal and injured dentate gyrus. The results show that early post-injury TLR4 antagonism alleviates long-term behavioral deficits and reveals adverse treatment outcomes in controls. Thus, selectively targeting the processes underlying pathological TLR4 signaling may be efficacious in reducing epilepsy and memory impairments after brain injury.

Disclosures: A.A. Korgaonkar: None. **J. Guevarra:** None. **K.C.H. Pang:** None. **V. Santhakumar:** None.

Poster

703. Non-Pharmacological and Molecular Strategies to Prevent Injury and Stroke.

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 703.10/Y17

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH Grant NS095441-01

AHA Grant 15SDG22760007

Title: High sodium diet increases Th17 differentiation and promotes neurovascular and cognitive dysfunction through IL-17 and Rho kinase-dependent eNOS inhibitory phosphorylation

Authors: *G. FARACO, D. BREA, G. WANG, G. RACCHUMI, H. CHANG, L. GARCIA-BONILLA, I. BUENDIA, K. KOIZUMI, J. ANRATHER, C. IADECOLA;
Brain and Mind Res. Inst., Weill Med. Col. of Cornell Univ., New York, NY

Abstract: High sodium diet (HSD) is thought to be a risk factor for stroke and dementia, independent of its effect on blood pressure (BP), but the mechanisms remain unclear. Therefore, we sought to investigate the cerebrovascular and cognitive impact of HSD. To this end, we fed HSD (4-8% NaCl) to C57BL/6 mice for 12 weeks and assessed cerebral blood flow (CBF) in the somatosensory cortex by laser-Doppler flowmetry. HSD did not increase BP (Control: 76.9±3.0mmHg; HSD: 76.3±1.6mmHg), but attenuated the CBF increase induced by neocortical application of acetylcholine (ACh) (-30.6±0.5%; $p<0.05$; n=8), a response mediated by endothelial nitric oxide synthase (eNOS), without affecting the response to the smooth muscle relaxant adenosine ($p>0.05$). These vascular changes were associated with a deficit in novel object exploration (Novel Object Exploration Time: ND 71±1.5%; HSD 54±1.8%; $p<0.05$; n=20), suggesting cognitive impairment. The impairment of the ACh response induced by HSD was associated with an increase in inhibitory eNOS phosphorylation (≈1.6 fold increase vs ND; $p<0.05$; n=7) in pial microvessels. Since HSD may lead to the expansion of intestinal helper T cells producing the vasotoxic cytokine IL17 (Th17 cell) (Nature, 496:518-22, 2013), we investigated whether IL-17 contributes to the effects of HSD. HSD increased the number of intestinal Th17 cells, assessed by flow cytometry (ND 211±45/cm; HSD 691±114/cm; $p<0.05$; n=8), as well as IL-17 plasma levels (ND 0.9±0.2pg/ml; HSD 6.5±1.4pg/ml; $p<0.05$; n=5). Consistent with a role of lymphocytes, the HSD-induced attenuation of the ACh CBF response was not observed in $Rag1^{-/-}$ mice, which lack both T and B lymphocytes. Furthermore, deletion of IL-17 or systemic administration of an IL-17 blocking antibody reduced plasma IL17, prevented inhibitory eNOS phosphorylation, and rescued the vascular and cognitive effects of HSD, whereas chronic injection of IL-17 reproduced fully the neurovascular effects of HSD. In brain endothelial cell cultures, IL-17 suppressed endothelial NO production in vitro, an effect associated with Rho-kinase-dependent increases in inhibitory eNOS phosphorylation. We conclude that HSD induces profound alterations in neurovascular regulation and cognitive function. The effect is mediated by expansion of intestinal Th17 cells, resulting in increases in circulating IL-17, which, in turn, lead to Rho kinase-induced inhibitory eNOS phosphorylation and reduced NO production. The data suggest a mechanism for the increased risk of stroke and dementia with HSD, and a putative therapeutic target for the deleterious effects of high salt on the brain.

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Poster

703. Non-Pharmacological and Molecular Strategies to Prevent Injury and Stroke.

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Program#/Poster#: 703.11/Y18

Topic: C.08.Stroke

Support: NIH Grant R01HD053727

Marquette University Research Leaders Fellowship

Title: Vibrotactile stimulation discrimination in the upper extremity of healthy humans

Authors: *V. SHAH[1], D. PETERS[1], M. GAGAS[1], A. KRUEGER[1,2], R. IANDOLO[3,2], M. CASADIO[2,3], R. A. SCHEIDT[1,4,5,6];
[1]Dept. of Biomed. Engin., Marquette Univ., Milwaukee, WI; [2]Dept. of Informatics, Bioengineering, Robotics and Systems Engin., Univ. of Genova, Genova, Italy; [3]Dept. of Robotics, Brain and Cognitive Sci., Italian Inst. of Technol., Genova, Italy; [4]Dept. of Physical Med. and Rehabil., Northwestern Univ. Feinberg Sch. of Med., Evanston, IL; [5]Sensory Motor Performance Program, Rehabil. Inst. of Chicago, Chicago, IL; [6]Dept. of Neurol., Med. Col. of Wisconsin, Wauwatosa, WI

Abstract: Proprioception is critical for effective planning and real-time control of movement. Unfortunately, proprioception can be impaired after stroke. Our long-term goal is to develop technologies that provide supplemental sensory feedback, via vibrotactile stimulation, to restore closed-loop control of the arm in stroke survivors who lack proprioceptive integrity. As a first step, we test the null hypothesis that vibrotactile stimulation of the different dermatomes within the arm give rise to similar perceptions (e.g., vibrotactile discrimination thresholds). The results of this study will allow us to determine whether some dermatomes are better suited than others as stimulation sites for providing supplemental kinesthetic feedback.
Eight neurologically intact subjects (age: 22 to 29 years) consented to participate in a two-alternative forced choice experiment. 10mm coin-motor “tactors” (Precision Microdrives, Ltd.) delivered vibratory stimuli at 5 different locations on the arm and hand (dermatomes C5, C7, C8, T1 and on the Ulnar Head, UH). Presentation of stimulus pairs conformed to the method of constant stimuli, wherein the standard vibratory stimulus was always presented at a frequency of 122 Hz. The probe stimulus could take on one of 10 different intensity values, spanning the range from 60 Hz to 172 Hz. 550 stimulus discrimination trials were presented in a single experimental session. Psychometric function estimation found the discrimination thresholds for the five locations to be: C5 = 22.7 Hz ± 11.7 (mean ± SEM), C7 = 16.3 Hz ± 11.8, C8 = 24.1 Hz ± 11.4, T1 = 27.7 Hz ± 11.0, and UH =19.4 Hz ± 10.9. Mixed model ANOVA found a significant difference in perception of vibrotactile stimuli across dermatomes. Post-hoc t-test found the discrimination threshold at C7 was significantly less than at T1 ($p=0.004$). Mean

discrimination thresholds at C5, C8, and UH did not differ from those at C7 or T1 ($p>0.05$ all cases).
As dermatome C7 had a significantly lower threshold than dermatome T1, we rejected the null hypothesis. Pacinian Corpuscles (PCs) which sense vibration on the skin, are sensitive to vibrations within the 50-350 Hz range (~300 Hz bandwidth). Thus, dermatome C7 can detect differences in stimulation frequency as small as ~5% of the PC full dynamic range. By contrast, C8 and T1 exhibited discrimination thresholds ~9% of the PC range. Thus, sensory augmentation and/or substitution via vibrotactile stimulation may exhibit constraints imposed by limited acuity of vibrotactile sensation. Future work should explore ways to enhance vibrotactile acuity in frequency discrimination and closed-loop control. Supported by NIH R01HD053727.

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Poster

703. Non-Pharmacological and Molecular Strategies to Prevent Injury and Stroke.

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 703.12/Z1

Topic: C.08.Stroke

Support: Doris Duke Charitable Foundation

VA Fellowship

Title: Resting state EEG power predicts peripheral neuromodulatory outcomes

Authors: N. NATRAJ, A. TSU, *K. GANGULY;
UCSF, San Francisco, CA

Abstract: Somatosensory peripheral nerve stimulation (PNS) is a promising method to promote motor recovery after stroke or brain injury. While past studies have indicated that there are significant effects of PNS on motor function, the underlying neural substrates are unclear. To this end, we conducted a pilot study to determine the neural correlates (via EEG) of PNS treatment on improvements in affected finger fractionation ability. Eight participants with a 6-month or greater history of acquired brain injury and distal upper limb motor impairment received a single two-hour session of submotor/suprasensory threshold stimulation using a transcutaneous electrical nerve stimulation (TENS) unit applied to the affected hand. Resting state EEG data over bilateral sensorimotor electrodes and finger fractionation ability were collected both pre and post PNS. PNS resulted in a significant drop in resting state low frequency power over

ipsilesional cortex at the group level, especially over ipsilesional motor cortex. Furthermore, the loss of ipsilesional motor cortex power was a significant predictor of individual fractionation improvements due to PNS in a ridge regression model. Results in this study warrant further investigations to optimize PNS for promoting hand dexterity recovery after acquired brain injury specifically targeting the aforementioned biomarker.

Disclosures: **N. Natraj:** None. **A. Tsu:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); intellectual property rights/patent holder. **K. Ganguly:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Intellectual property rights/patent holder.

Poster

703. Non-Pharmacological and Molecular Strategies to Prevent Injury and Stroke.

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 703.13/Z2

Topic: C.08.Stroke

Support: RSF grant 14-25-000

Title: Morphology of neurons in penumbra area of ischemic insult after lentiviral delivery of neurotrophins to the rat brain cortex.

Authors: *G. SMIRNOVA[1], M. SVINOV[2];
[1]Cell. Neurobio. of Learning Lab., [2]Functional Neurocytology Lab., Inst. of Higher Nervous Activity and Neurophys, Moskva, Russian Federation

Abstract: Therapeutic approaches in ischemic insult treatment include wide range technologies. One of these technologies is lentiviral delivery of neurotrophins. The popular sites of delivery are brain ventricles. And the main measure of such treatment effectiveness is volume of infarct size In the present study, we examined the influence of neurotrophins on the morphology of neurons and astrocytes in ischemic penumbra. We injected lentiviral suspension in cerebral cortex 2 weeks before photothrombotic insult in rats. On the second day after insult rats were sacrificed, parallel brain slices were stained by Toluidine Blue or by antibodies to GFP, GFAP, and DAPI. The area of parenchymal delivery of neurotrothins was overlapped with the area of ischemic penumbra. In this overlapping area we analyzed morphology of neurons. The neurotrothins injection leads to decrease of dark pyramidal neurons in V layer of cerebral cortex.

Disclosures: **G. Smirnova:** None. **M. Svinov:** None.

Poster

703. Non-Pharmacological and Molecular Strategies to Prevent Injury and Stroke.

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 703.14/Z3

Topic: C.08.Stroke

Support: Swedish Research Council

Maggie Stephens Stiftelse

Lund University Medical Faculty

STROKE-Riksförbundet

Kungliga Fysiografiska Sällskapet i Lund

MultiPark

Läkaresällskapet i Lund

Title: Extracellular matrix structures and modulators in the rat somatosensory cortex: molecular substrates for experience-dependent plasticity during stroke recovery

Authors: *M. QUATTROMANI[1], M. PRUVOST[2], C. GUERREIRO[1], F. BACKLUND[1], K. RUSCHER[1], D. VIVIEN[2], T. WIELOCH[1];
[1]Lund Univ., Lund, Sweden; [2]GIP Cyceron / Univ. of Caen, Caen, France

Abstract: Stroke can be conceived as a site of complete cellular death surrounded by tissue that undergoes reorganization. Experience-dependent plasticity exerted through an enriched environment (EE) promotes neuronal remodeling and neuroplasticity after central nervous system injury (i.e. stroke). After stroke, a number of growth-inhibitory molecules are differentially expressed within the ischemic hemisphere, leading to inhibition of neuronal plasticity. Particular interest has been focused on the inhibitory role of chondroitin sulfate proteoglycans (CSPGs) and their formation into dense lattice-like structures, termed perineuronal nets (PNNs). PNNs enwrap sub-populations of neurons, such as parvalbumin-containing GABAergic (PV/GABA) positive cells, important in sensori-information processing. Our recent study (Madinier and Quattromani et al., 2014) demonstrates that during stroke recovery, EE induces a reduction in the number of PNNs in peri-infarct and regions remote from the infarct. The objective of this study was to investigate if extracellular matrix (ECM) modulators such as metalloproteinases (MMPs), especially MMP-2 and MMP-9, tissue plasminogen activator (tPA) and type 4 disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS-4) may participate in the degradation of PNNs that we observe after stroke and EE conditions. Male

Sprague Dawley rats were subjected to photothrombotic stroke (PT) in the motor cortex and functional deficits assessed at 2 and 7 days of recovery. Shams and stroked rats were housed in either standard (STD) or EE conditions for 5 days and infarct volumes calculated. Aggrecan-containing PNNs were visualized by immunohistochemistry (Cat-315$^+$ neurons) and immunofluorescence (Cat-315/PV$^+$ neurons) and counted in bright-field and confocal microscopy in the somatosensory cortex of both hemispheres. PNN protease mRNA levels were assessed by qRT-PCR and their activity analyzed by real-time-FITC-gel zymography and casein-plasminogen zymography. EE starting 2 days after PT and continuing for 5 days did not influence the size of the infarct, but induced a remarkable difference in behavioral recovery between the housing groups. A reduction in the number of Cat-315$^+$ and Cat-315/PV$^+$ neurons was observed in the somatosensory cortex of the ipsilateral hemisphere after PT and EE and was accompanied by modulation in the expression and activity of ECM proteases. Elucidating the mechanisms and pharmacology of CSPGs and ECM turnover after experimental stroke may then provide new therapies supporting rehabilitation.

Disclosures: M. Quattromani: None. **M. Pruvost:** None. **C. Guerreiro:** None. **F. Backlund:** None. **K. Ruscher:** None. **D. Vivien:** None. **T. Wieloch:** None.

Poster

703. Non-Pharmacological and Molecular Strategies to Prevent Injury and Stroke.

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 703.15/Z4

Topic: C.08.Stroke

Support: Disability Communications Fund

Casa Colina Foundation

Title: The effects of an augmentative and alternative communication intervention on functional communication and neural activity patterns in individuals with aphasia

Authors: *S. ROSENBERG, M. LAM, J. DIVINE, H. MILLAN, N. FULLMER;
Res. Inst., Casa Colina Hosp. and Centers for Healthcare, Claremont, CA

Abstract: Aphasia is a communication disorder caused by neurological damage, which can impair one's ability to understand and use both written and spoken language. Annually, 80,000 individuals in the United States will acquire aphasia as a result of a stroke. The purpose of this pilot study was to evaluate the effect of an augmentative and alternative communication (AAC) program on the functional communication abilities and neural activity patterns of individuals

with aphasia. Individuals (n = 20) enrolled in the study were between the ages of 18 to 90 and were at least 3 months post-onset of aphasia. Participants attended six one-hour therapy sessions with a certified speech and language pathologist to work on their communication abilities using the AAC program. Participants completed pre-evaluations prior to the intervention sessions and post-evaluations approximately one week after completion of the intervention. Evaluations included the Western Aphasia Battery (WAB), which measures expressive and receptive communication capabilities. Results from the study indicate improvements on the WAB following completion of the AAC intervention. Participants also completed a prompt and response task in which prompts involved verbal commands to arrange provided items in a specific manner. Verbal commands were provided alone or were accompanied with static or dynamic visual cues. Preliminary data from the prompt and response evaluation show a statistically significant difference between the ability to respond correctly to verbal only prompts as compared to verbal prompts with visually augmentative cues. Pre/post intervention evaluations also included electroencephalogram (EEG) recordings to assess the potential for use of wireless EEG recordings during clinical outcome measures. A 24-channel wireless EEG system was used to record neural activity patterns during both the prompt/response task and during an eyes open/eyes closed paradigm. Preliminary analysis of the resting state EEG recordings indicates the presence of relative power spectral density patterns similar to those of previous studies conducted with individuals with aphasia. Furthermore, these pilot recordings support the feasibility of conducting EEG recordings during clinical outcome measurements. The results of this pilot study suggest the potential for assessing correlations between neural activity patterns and functional communication abilities and provide support for the idea that supplementing verbal commands with visual aids and utilizing AAC interventions with visual cues may help enhance the communication abilities and daily lives of individuals with aphasia.

Disclosures: S. Rosenberg: None. **M. Lam:** None. **J. Divine:** None. **H. Millan:** None. **N. Fullmer:** None.

Poster

703. Non-Pharmacological and Molecular Strategies to Prevent Injury and Stroke.

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Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 703.16/Z5

Topic: C.08.Stroke

Support: PJ010508042015

Title: Human cerebral endothelial cell transplantation in a rat model of cerebral ischemia for neuroprotection

Authors: S. YU[1], H.-W. RYU[1,3], J.-T. PARK[2], *H. KIM[2,3];
[1]Forensic Med., [2]Chonnam Natl. Univ. Med. Sch., Gwangju, Korea, Republic of; [3]Ctr. for Creative Biomed. Scientists at Chonnam Natl. Univ. Med. Sch., Gwangju, Korea, Republic of

Abstract: Human cerebral microvascular endothelial cell line (hCMEC)/D3 cells, which are from a stable clonal cell line of human immortalized cerebral endothelial cells, were intra-arterially transplanted through the common carotid artery in a rat model of photochemical-induced cerebral ischemia. Their therapeutic effects on infarct size, blood-brain barrier (BBB) breakdown, and outcome were examined. The hCMEC/D3 cells were genetically modified with the firefly luciferase gene for in vivo imaging post-transplantation. Transplanted hCMEC/D3 cells were identified in the infarcted brain by bioluminescence imaging at 1 day after transplantation. Compared with the control group, the hCMEC/D3-transplanted group showed reduced infarct size on day 3, reduced Evans blue dye leakage on day 1 indicating decreased BBB breakdown, and early recovery from Rotarod test neurological deficits. The hCMEC/D3-transplanted group also showed decreased levels of matrix metalloproteinase (MMP)-9, which were inversely correlated with TIMP-1 levels on post-transplantation days 1 and 3. The expression of tumor necrosis factor-α and interleukin-1β were markedly diminished in the hCMEC/D3-transplanted group compared with controls. The systemically transplanted cells selectively migrated and integrated into the ischemically lesioned area, which accelerated neurological recovery. This new cerebral endothelial cell-based therapy may hold promise for clinical trials in patients with ischemic stroke. ** This work was equally distributed. (Financial supports: This work was carried out with the support of the “Cooperative Research Program for Agriculture Science & Technology Development (Project title: Diversification of food materials for new demands in domestic Oat, Project No. PJ010508042015)” from the Rural Development Administration) and Center for Creative Biomedical Scientists at Chonnam National University Medical School, Gwangju, Republic of Korea.

Disclosures: S. Yu: None. **H. Ryu:** None. **J. Park:** None. **H. Kim:** None.

Poster

703. Non-Pharmacological and Molecular Strategies to Prevent Injury and Stroke.

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 703.17/Z6

Topic: C.08.Stroke

Support: NIH Grant NS088413

NIH Grant NS085568

NIH Grant NS091585

Title: Altered autophagy activity after ischemic stroke in GPR37 knockout mice

Authors: *M. R. MCCRARY[1], M. Q. JIANG[2], J. Y. ZHANG[2], X. GU[2], M. GIDDENS[2], R. HALL[3], S. P. YU[4], L. WEI[4];
[1]Biomed. Engin., [2]Neurosci., [3]Pharmacol., [4]Anesthesiol., Emory Univ., Atlanta, GA

Abstract: Stroke is a debilitating brain injury leading to mortality and neurological disability. GPR37 is a G protein-coupled receptor that has been found to exert neuroprotective and glioprotective effects in vitro, but the relationship between GPR37 and mechanisms of cell survival or death remain unclear. Autophagy plays a dual role in promoting cell survival via digestion and recycling of cellular components, and also contributes to cell death by lysosomal processes of self-induced intracellular degradation. Autophagic cell death has been implicated in secondary damage after ischemic stroke. In the present investigation, we tested a possible role of GPR37 signaling in regulating autophagy in the ischemic brain using wild type (WT) and GPR37 knockout (GPR37 KO) mice. Adult mice were subjected to focal cerebral ischemia targeting the sensorimotor cortex. Infarct volume was measured using 2,3,5-triphenyltetrazolium chloride (TTC) staining 3 days after stroke. Images of stained slides were analyzed using ImageJ under double-blind conditions. Stroke animals were sacrificed at 6, 12, 24, and 48 hours after stroke for Western blot analyses. Tissue was isolated from the infarct core, the peri-infarct regions, and the contralateral cortex. TTC measurement revealed that GPR37 KO mice experience significantly enlarged infarct volumes compared to WT stroke controls ($p=0.0424$). To evaluate autophagy activity, we measured the expression of Beclin-1, a primary component of the pre-autophagosomal structure, and microtubule-associated protein 1 light chain 3 (LC3), an effector of autophagy. Compared to WT controls, Beclin-1 levels in GPR37 KOs were increased in the peri-infarct area at 6 and 12 hours post-stroke. LC3 levels were also increased in the GPR37 KOs at 12 hours after stroke. The anti-apoptotic gene Bcl-2 was differently regulated. Taken together, these data suggest that autophagy is upregulated in the peri-infarct region of GPR37 KO mice, raising the possibility that GPR37 plays a role in regulating apoptosis and/or autophagy. Future studies will elucidate the complex interactions between autophagy, programmed cell death, and GPR37 signaling in ischemic stroke.

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Poster

703. Non-Pharmacological and Molecular Strategies to Prevent Injury and Stroke.

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 703.18/Z7

Topic: C.09. Brain Injury and Trauma

Support: NIH Grant 5T32GM008620

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Generous private donations

Title: Adult deletion of PTEN in cortical motoneurons leads to robust cell body enlargement that is maintained as the steady-state cell size

Authors: *E. A. GUTILLA, O. STEWARD;
UC Irvine, Irvine, CA

Abstract: Adult neurons often undergo atrophy following axonal injury, in neurodegenerative diseases and with aging; such atrophy is thought to reflect decreased vitality. Neurons in which *PTEN* has been deleted are enlarged one year post-deletion, but it is not known if there is a transient growth response after *PTEN* deletion that results in a larger steady-state cell size, whether growth continues progressively as long as *PTEN* is absent, or whether deletion of *PTEN* prevents age-related neuronal atrophy. To address this question, we assessed changes in neuron size with time following deletion of *PTEN* in the sensorimotor cortex of adult (8 week old) mice. To positively identify neurons in which *PTEN* was deleted, we used double transgenic mice with a lox-P flanked exon 5 of the *PTEN* gene and lox-P flanked STOP cassette that regulates expression of tdTomato. Cre-mediated recombination leads to deletion of *PTEN* and expression of tdTomato in the same neurons. Mice received unilateral intracortical injections of AAV-Cre, and were allowed to survive for 3, 4, 5, 6, 9, or 12 months before being given bilateral injections of fluorogold at cervical level 5 of the spinal cord to retrogradely label the cortical motoneurons (CMNs) that give rise to the corticospinal tract. Retrogradely-labeled CMNs expressing tdTomato (also *PTEN*-negative) were compared with retrogradely-labeled CMNs in the contralateral cortex. Quantitative assessments revealed that cell bodies of CMNs lacking *PTEN* are 1.3 times the size of control CMNs by 4 months after the deletion. CMNs lacking *PTEN* remained significantly enlarged at all time points beyond 4 months but the magnitude of the differences between CMNs lacking *PTEN* and contralateral controls did not increase further after 4 months. Immunostaining for the phosphorylated form of ribosomal protein S6 (a biomarker for activation of the mammalian target of rapamycin, or mTOR, pathway) revealed continued activation at all time points after *PTEN* deletion up to 12 months. These results indicate that deleting *PTEN* in adult neurons triggers robust neuronal growth in which neurons enlarge to a

new steady-state size that is maintained throughout life. These findings suggest novel strategies to prevent neuronal atrophy due to aging, traumatic injury, or neurodegenerative disease.

Disclosures: E.A. Gutilla: None. **O. Steward:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Co-founder of Axonis.

Poster

703. Non-Pharmacological and Molecular Strategies to Prevent Injury and Stroke.

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 703.19/Z8

Topic: C.09. Brain Injury and Trauma

Support: Niemann-Pick Research Foundation UK

Asociación Niemann Pick de Fuenlabrada

Internal UCL Funding

Title: Development of gene therapy for Niemann-Pick Type C disease

Authors: *M. HUGHES[1], D. SMITH[2], L. MORRIS[2], J. TORDO[3], N. PALOMAR-MARTIN[3], E. HENCKAERTS[3], S. WADDINGTON[4], F. PLATT[2], A. RAHIM[1];
[1]Sch. Of Pharm., UCL, London, United Kingdom; [2]Dept. of Pharmacol., Univ. of Oxford, Oxford, United Kingdom; [3]Dept. of Infectious Dis., King's Col. London, London, United Kingdom; [4]Inst. For Women's Hlth., Univ. Col. London, London, United Kingdom

Abstract: Niemann-Pick type C is a lysosomal storage disorder with neurological and visceral pathology, for which there is currently no major disease modifying treatment. Loss of NPC1 function, a late endosomal transmembrane protein, leads to systemic intracellular lipid accumulation. However, premature death is associated with neurological manifestations, such as severe neurodegeneration and neuroinflammation. Advances in viral vector technology and recent successful clinical trials display the potential beneficial use of gene therapy for these types of monogenic disorders.
This project focuses on the development of an adeno-associated viral (AAV) vector capable of delivering and expressing human *NPC1* in the mouse brain, via perinatal intracranial or intravenous injection, with the ultimate aim of ameliorating brain and visceral pathology in the *Npc1*$^{-/-}$ mouse model. AAV vectors exhibit efficient and widespread gene delivery throughout the brain, however their limited packaging capacity of 4.7kb can be a constraint for larger genes. Extensive construct modifications were made to incorporate the relatively large 3.8kb *NPC1*

gene into a functional AAV serotype 9 vector. *NPC1* expression is controlled by the constitutively active neuronal promoter human *synapsin I*, which in combination with an AAV9 vector produces impressive levels of gene delivery and neuronal specificity, following administration to the CNS.
Initial *in vivo* tests demonstrated successful NPC1 over-expression in administered mouse brains, compared to endogenous Npc1 levels in un-administered controls. No indications of toxicity or neuroinflammation were observed as a result of NPC1 over-expression *in vivo*. A preliminary very low dose study was carried out on the *Npc1*$^{-/-}$ model, where newborn *Npc1*$^{-/-}$ mice were administered with $4.6x10^9$ vector genomes of AAV9.SYN1.NPC1 via intracranial injections. Treated *Npc1*$^{-/-}$ mice exhibited an increased lifespan (n=8; median survival of 116.5 days; $P<0.0001$) compared to untreated Npc1-/- mice (n=6; median survival of 75.5 days). Combined with significant behavioural improvements these results demonstrate the potential beneficial use of gene therapy for Niemann-Pick type C and support the continuation of this approach with higher dose studies.

Disclosures: **M. Hughes:** None. **D. Smith:** None. **L. Morris:** None. **J. Tordo:** None. **N. Palomar-Martin:** None. **E. Henckaerts:** None. **S. Waddington:** None. **F. Platt:** None. **A. Rahim:** None.

Poster

703. Non-Pharmacological and Molecular Strategies to Prevent Injury and Stroke.

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Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 703.20/Z9

Topic: C.09. Brain Injury and Trauma

Support: UCL Impact Studentship

UK Gauchers Association

Title: Intravenously administered gene therapy for the treatment of Gaucher Disease.

Authors: ***G. MASSARO**[1], D. PEROCHEAU[2], R. BAKER[3], S. KARLSSON[4], S. H. CHENG[5], S. N. WADDINGTON[2], A. A. RAHIM[1];
[1]Sch. of Pharm., UCL, London, United Kingdom; [2]UCL Inst. for Women's Hlth., London, United Kingdom; [3]Royal Free Site, HSL LLP, London, United Kingdom; [4]Lund Univ., Lund, Sweden; [5]Genzyme, A Sanofi Co., Framingham, MA

Abstract: Gaucher Disease is caused by mutations in the *GBA* gene encoding the lysosomal enzyme glucocerebrosidase (GCase). Deficiency of GCase causes the accumulation of

glucosylceramide in enlarged “Gaucher cells” within visceral organs and brain. Recombinant human enzyme is commercially available and is successfully used to treat the visceral pathology in non-neuronopathic Gaucher Disease patients. Acute neuronopathic Gaucher Disease (nGD) is characterised by neuronal loss, astrocytosis and microglial proliferation. nGD is untreatable since enzyme replacement therapy cannot cross the blood-brain barrier.
In this study, I tested the hypothesis that neonatal intravenous injection of adeno-associated viral vector serotype 9 (AAV9), carrying functional *GBA* gene, would improve lifespan, behavior, brain and visceral pathology in a mouse model of nGD.
AAV9 has been demonstrated to be able to efficiently transduce the Central Nervous System and visceral organs following intravenous administration to mice and non-human primates.
Untreated knock-out mice die 12-14 days after birth. Treated mice showed a >10-fold increase in their lifespan. Neuropathological markers such as microglia-mediated inflammation, astrogliosis and lysosomal accumulation were ameliorated and some of the most affected areas of the brain, like thalamus, brain stem and cerebellum were partially rescued. Histologic analysis, enzymatic assay and blood test revealed improvement in the visceral pathology. In the lung, spleen and liver the presence of Gaucher cells is significantly reduced and tissue architecture is preserved.

Disclosures: G. Massaro: None. **D. Perocheau:** None. **R. Baker:** None. **S. Karlsson:** None. **S.H. Cheng:** None. **S.N. Waddington:** None. **A.A. Rahim:** None.

Poster

704. Ischemia: Inflammation

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 704.01/Z10

Topic: C.07. Ischemia

Title: Stroke affects intestinal immune cell trafficking to the CNS

Authors: *D. BREA[1], C. BENAKIS[2], M. MURPHY[2], C. IADECOLA[2], J. ANRATHER[2];
[1]Weill Cornell Med. Col., Joan and Sanford I Weill Med. Col. of Cornell Univ., New York, NY;
[2]Weill Cornell Med., New York, NY

Abstract: Stroke is an acute neurological disease with a strong inflammatory component that can be regulated at the intestinal level by mediation of intestinal microbiota and intestinal immune cells (Nature Medicine 22, p. 516, 2016). Although stroke has been shown to alter immune cell populations in the gut (Neuroimmunomodulation 16, p. 213, 2009), the dynamics of cell trafficking have not been elucidated. To study the trafficking of gut-derived immune cells, we used mice expressing the photoconvertible protein Kikume Green-Red. Mice underwent laparotomy and the small intestine (6 cm) was exposed to violet light (405 nm) using a laser

source. Immune cells were isolated from the small intestine immediately, 2, 7 and 14 days after photoconversion. Percentage of immune cells ($CD45^{hi}/KikR^{+}$) that expressed the red variant of the protein (KikR) was higher at days 0 (37.76±1.11%) and 2 (35.03±1.15%) than at days 7 (11.60±1.77%) and 14 (4.87±0.37%) in the intestinal segment exposed to violet light. Extrapolating these results to the total intestinal immune cell population, we calculated that 5.9±0.8% of gut immune cells were expressing KikR at day 2 after photoconversion. To investigate whether intestinal immune cells traffic to the periphery after stroke, we analyzed KikR+ immune cells (2 days after photoconversion) in mesenteric and cervical lymph nodes, spleen, bone marrow, meninges and brain, 3 and 14 days after tMCAo. Gut-derived KikR+ immune cells were found in all analyzed organs at 3 and 14 days after stroke or sham surgery. KikR+ cell numbers and frequencies were similar in peripheral organs both at 3 and 14 days after stroke. The number of KikR+ immune cells was higher in brains 3 and 14 days after stroke when compared to sham animals (3 days: 187 ± 133 vs. 5 ± 3 cells/hemisphere, n=5-7, $p<0.01$; 14 days: 155 ± 102 vs. 3 ± 1 cells/hemisphere, n=5-6, $p < 0.01$). Numbers of meningeal KikR+ immune cells in sham animals were higher than in the brain indicating the presence of gut-derived immune cells under homeostatic conditions. Meningeal KikR+ immune cells were not significantly altered 3 or 14 days after stroke. KikR+ immune cells were found in all lymphoid organs analyzed but were not affected by stroke. We conclude that intestinal immune cells traffic to peripheral lymphoid organs and meninges under homeostatic conditions and this behavior is not affected 3 and 14 days after tMCAo, while gut-derived immune cells are increased in the brain at 3 and 14 days after stroke. Our data suggest stochastic intestinal immune cell trafficking under homeostatic conditions and a directed recruitment to sites of sterile inflammation such as ischemic brain injury.

Disclosures: **D. Brea:** None. **C. Benakis:** None. **M. Murphy:** None. **C. Iadecola:** None. **J. Anrather:** None.

Poster

704. Ischemia: Inflammation

Location: Halls B-H

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Program#/Poster#: 704.02/Z11

Topic: C.07. Ischemia

Support: NIH Grant F30-NS092168

NIH Grant R01-NS076617

Schmitt Program on Integrative Brain Research

Title: Systemic immune responses and neutrophil activation in the propagation of neuroinflammation after cerebral ischemia-reperfusion

Authors: *N. MAI, L. PRIFTI, M. W. HALTERMAN;
Univ. of Rochester, Rochester, NY

Abstract: Post-ischemic neuroinflammation remains an elusive therapeutic target for patients suffering from global cerebral ischemia following cardiac arrest. While heightened inflammatory responses portend poor neurologic recovery, the lack of pre-clinical models that faithfully reproduce features of post-cardiac arrest syndrome (PCAS) remains a barrier to developing effective neuroprotective therapies. Here we demonstrate that serologically undetectable endotoxemia causes acute, peripheral neutrophil activation and enhanced CNS inflammation when combined with global cerebral ischemia.
Transient global ischemia was induced in 5-8 week-old C57/B6 mice using the 3-vessel occlusion (3VO) model, which involves basilar artery cauterization followed by 15-minute occlusion of the common carotid arteries. During reperfusion, mice were given saline or 50 μg/kg lipopolysaccharide (LPS) intraperitoneally to mimic enteric endotoxin leak and systemic inflammatory responses observed during PCAS. Animals receiving saline or LPS served as sham controls.
While 50 μg/kg LPS yielded undetectable endotoxin levels in serum 6 hours after injury, upregulation of the neutrophil activation marker CD11b was observed in sham-treated animals, with greater activation in those also undergoing 3VO. Three days after ischemia-reperfusion, animals with concomitant systemic inflammation experienced the greatest blood-brain barrier permeability, as evidenced by upregulation of vascular platelet endothelial cell adhesion molecule-1, enhanced perivascular IgG deposition, and neutrophil migration into the brain parenchyma. Animals receiving 3VO and LPS exhibited pronounced microglial activation characterized by Iba1 upregulation and amoeboid morphology. These phenotypic changes were specific to areas of dense neutrophil infiltration, suggesting that the effects were likely cell-mediated and not a consequence of passive endotoxin transfer across the blood-brain barrier. While the role of neutrophils in cerebral ischemia reperfusion injury remains controversial, our data indicate that neutrophil activation and migration remain particularly relevant targets for mitigating neuroinflammation in the setting of cardiac arrest.

Disclosures: **N. Mai:** None. **L. Prifti:** None. **M.W. Halterman:** None.

Poster

704. Ischemia: Inflammation

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Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 704.03/Z12

Topic: C.07. Ischemia

Support: NIH/NINDS research grant R01 NINDS00088

Title: Anti-inflammatory role of g-protein coupled receptor 30 (gpr30) in the brain following global cerebral ischemia

Authors: *R. WANG, D. BRANN;
Dept. of Neurosci. and Regenerative Med., Augusta Univ., Augusta, GA

Abstract: The NLRP3 inflammasome is now recognized to have an important role in neuroinflammation and delayed neuronal death following ischemic injury to the brain. G-Protein Coupled Receptor 30 **(**GPR30) is a seven trans-membrane protein and is purported to function as an estrogen receptor to help mediate estrogen signaling and actions in the body. In the current study, we examined whether GPR30 may exert an anti-inflammatory regulatory effect to inhibit NLRP3 inflammasome activation and signaling following global cerebral ischemia (GCI). A GPR30 agonist (G1) or antagonist (G36) were administrated to ovariectomized female rats by minipump beginning at 1d after ischemic reperfusion and continued until 7d or 14d of reperfusion. The following endpoints were examined: 1) cell type localization of GPR30 in the hippocampal CA1 region, 2) protein levels of NLRP3, ASC, TXNIP, and caspase 1, which are essential components of the NLRP3 inflammasome pathway, 3) expression of IL1RA, an antagonist of the Interleukin 1 receptor (IL-1R). The results showed that GPR30 was expressed in neuron in the sham (non-ischemic) group, however at 7d and 14d reperfusion, GPR30 was mainly expressed in reactive astrocytes, with some expression also noted in microglia. Immunofluorescence staining of NLRP3, ASC, TXNIP, Caspase 1 showed that protein levels of the inflammasome components significantly increased at reperfusion 7d and 14d. Simultaneously, the levels of cleaved IL1β were markedly enhanced in ischemia groups, compared to the sham control. Furthermore, there was a concomitant decrease of IL1RA protein levels following GCI. Importantly, post-ischemic administration of the GPR30 agonist, G1, enhanced GPR30 protein levels in hippocampal neurons and was neuroprotective against GCI. G1 treatment also attenuated the GCI-induced elevation of inflammasome components and cleaved IL1β, and reversed the GCI-induced decrease of IL1RA. Finally, continuous administration of G36, a GPR30 specific antagonist, prevented the G1 anti-inflammatory effects at 7d and 14d after GCI. Taken together, the current results suggest that GPR30 signaling can inhibit NLRP3 inflammasome activation after GCI, and exerts potent anti-inflammatory and neuroprotective effects in the hippocampus.

Disclosures: R. Wang: None. **D. Brann:** None.

Poster

704. Ischemia: Inflammation

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Program#/Poster#: 704.04/Z13

Topic: C.07. Ischemia

Title: Immunization with Cop-1 induces changes in gene expression at the choroid plexus of rats subjected to focal cerebral ischemia reperfusion.

Authors: *Y. CRUZ[1,2], E. GARCÍA[1], T. RODRÍGUEZ[1], E. STA.MARÍA[1], H. BONILLA[2], J. ROJAS[3], V. GÁLVEZ[1], K. CANTÚ[1], G. OJEDA[1], A. IBARRA[1];
[1]Univ. Anáhuac, México, Mexico; [2]Univ. Autónoma Metropolitana Unidad Iztapalapa, Ciudad de México, Mexico; [3]Inst. Nacional de Pediatría, Ciudad de México, Mexico

Abstract: Cerebrovascular diseases are pathologies caused by alterations in blood flow. Cerebral ischemia results in events such as excitotoxicity, necrosis, apoptosis and inflammation, leading to neuronal death. Immunization with neural-derived peptides such as Copolymer-1 (Cop-1) has demonstrated to provide neuroprotective and neurogenic effects in a cerebral ischemia model, thus preserving tissue, and promoting better motor recovery and neurogenesis.
Cop-1 modulates the immune response through the induction of lymphocyte differentiation towards a Th-2 phenotype during the event; these Th-2 lymphocytes modulate the production of anti-inflammatory cytokines and trophic factors that promote neural tissue restoration.
Recent studies have shown that physiological communication between the immune and nervous system takes place in the choroid plexus (CP) through cytokines and neurotrophic factors. This microenvironment determines processes such as neurogenesis and brain plasticity; for this reason, it was analyzed whether Cop-1 immunization can modify gene expression of cytokines and neurotrophic factors at the CP and if this change correlates with neurogenesis after ischemia.
Cytokine (IFNγ, TNFα, IL-1β, IL-17, IL-4) and neurotrophic factor (IGF-1 and neurotrophin 3 (NT-3)) gene expression was evaluated in rats subjected to cerebral ischemia and immunized with: Cop-1+complete Freund's adyuvant (CFA), Cop-1 + Saline and CFA+ Saline; non-immunized rats were used as controls. Neurogenesis was evaluated at the subventricular zone, hippocampal dentate gyrus, and cerebral cortex by immunofluorescence using the following markers: BrdU (bromodeoxyuridine) and DCX (doublecortin).
Seven days after ischemia, immunization with Cop-1+ CFA induced a reduction in the expression of IFNγ, TNFα, IL-1β, IL-4, IGF-1 and NT-3 genes. Fourteen days later, the expression of these genes increased; however, the gene encoding for IL-4 remained reduced. Neurogenesis was significantly increased at 7 and 14 days in the group treated with Cop-1 + CFA.
These results demonstrate that Cop-1 has the ability of modulating the production of cytokines

and neurotrophic factors at the CP. These changes could be involved in the process of neurogenesis.

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Poster

704. Ischemia: Inflammation

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Topic: C.07. Ischemia

Support: AHA grant 12SDG8170005 to JY

Title: Amelioration of cerebral ischemic stroke by induction of Nrf2/HO-1 pathway

Authors: ***P.-C. KUO**[1], B. A. SCOFIELD[1], D. A. BROWN[2], J.-H. YEN[1];
[1]Dept. of Microbiology and Immunol., Indiana Univ. Sch. of Med., Fort Wayne, IN; [2]Dept. of Pharmaceut. Sci., Manchester Univ. Col. of Pharm., Fort Wayne, IN

Abstract: Cerebral ischemic stroke is involved in over 80% of all stroke cases. During cerebral ischemia, reactive oxygen species (ROS) is produced in brain tissue, which can then initiate numerous signaling pathways and cause oxidative stress and inflammatory response. Studies suggest that both oxidative stress and inflammatory damage are essential pathological mechanisms in cerebral ischemia. To date, recombinant tissue plasminogen activator (tPA) is the only available therapy for the treatment of ischemic stroke; however, the treatment does not prevent ischemia-induced oxidative stress and reperfusion-mediated inflammation in ischemic brains. Therefore, the development of new anti-oxidant and anti-inflammatory therapies for the treatment of ischemic stroke is necessary and urgent. Nuclear factor erythroid 2-related factor 2 (Nrf2), a major regulator of oxidative responses, is a key transcription factor which induces a wide set of anti-oxidant enzymes and phase II detoxification enzymes, including heme oxygenase 1 (HO-1) and NAD(P)H dehydrogenase, quinone 1 (NQO1). In addition, Nrf2 exerts an anti-inflammatory effect through HO-1/NF-κB pathway. Our lab has identified a small molecule (BY-23) with a property of inducing Nrf2 activation, and we evaluated its therapeutic potential for the treatment of ischemic stroke in the animal model of transient middle cerebral artery occlusion/reperfusion (tMCAO/R). Our results show BY-23 treatment not only ameliorated neurological deficits in ischemic stroke mice but dramatically reduced infract size in ischemic brains. We also observed significant reduction of CNS infiltrating cells including

neutrophils and monocytes in the ischemic brain of BY-23-treated when compared to those of vehicle-treated tMCAO/R mice. At the molecular level, BY-23 treatment enhanced the expression of anti-oxidant enzymes HO-1 and NQO1 in the ischemic brains of tMCAO/R mice. In the presence of BY-23, the expression of Nrf2, HO-1, NQO1, and glutamate-cysteine ligase, catalytic subunit (GCLC) was significantly upregulated in LPS-activated microglia (MG). Importantly, BY-23 suppressed MG activation in the ischemic brain of tMCAO/R mice, and the protective effect of BY-23 on attenuating brain infarct in ischemic stroke was abolished in the presence of HO-1 inhibitor. Altogether, our results suggest that BY-23-activated Nrf2/HO-1 pathway confers a protective effect against ischemic stroke.

Disclosures: P. Kuo: None. **B.A. Scofield:** None. **D.A. Brown:** None. **J. Yen:** None.

Poster

704. Ischemia: Inflammation

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Program#/Poster#: 704.06/AA1

Topic: C.07. Ischemia

Title: Prophylactic chronic administration of zinc at tolerable doses increases the expression of chemokines and receptors in a rat model of cerebral hypoxia ischemia

Authors: *C. T. SANCHEZ[1], V. M. BLANCO-ALVAREZ[2], J. A. GONZALEZ-BARRIOS[4], D. MARTINEZ-FONG[5], G. GARCIA-ROBLES[2], G. SOTO-RODRIGUEZ[5], E. BRAMBILA-COLOMBRES[3], B. A. LEON-CHAVEZ[3];
[1]Benemerita Univ. Autonoma De Puebla, Puebla, Mexico; [2]Posgrado en Ciencias Quimicas, Benemerita Univ. Autonoma de Puebla, Puebla, Mexico; [3]Posgrado en Ciencias Quimicas, Benemerita Univ. Autonoma de Puebla, Mexico city, Mexico; [4]Lab. de Medicina Genomica, ISSSTE, mexico city, Mexico; [5]Fisiologia, Biofisica y Neurosciencias, Ctr. de investigaciones y Estudios Avanzados, Mexico city, Mexico

Abstract: Zinc administration might be used as a prophylactic agent in cerebrovascular disease in the CNS, because acute and subacute administration of zinc is known to exert neuroprotection in hypoxia-ischemia animal models. However, chronic administration of zinc is cytotoxic. The effect of prophylactic chronic administration of zinc on neuroinflammation is still unknown. To clarify this issue, we administered the zinc at tolerable doses,0.5 mg/kg $ZnCl_2$ every 24 h for 14 days, to rats before a common carotid artery occlusion, CCAO. After CCAO, the level of zinc was measured by atomic absorption spectrophotometry, nitrites were determined by Griess method, lipoperoxidation by Gerard-Monnier assay, mRNA expression of 84 genes coding for cytokines, chemokines and their receptors by qRT-PCR (Qiagen, Inc), whereas nitrotyrosine,

chemokines and their receptors were assessed by ELISA and histopathological changes in the temporoparietal cortex-hippocampus at different time points (0 h, 8 h and 7 days). We found that only zinc administration in control rats increases inflammatory chemokines/receptors such as CXCL12, CXCL5 CCL4 and the growth factors FGF2 and VEGF that would be associated with a preconditioning process. In addition, zinc administration in rats with CCAO also increased CCL20, CXCL5, CCR2, CCR4, CCR6, CCR8 and CXCR2 levels at 8 h and 168 h post-reperfusion, and FGF2 and VEGF at 168 h following CCAO. This data show that the prophylactic chronic administration of zinc at tolerable doses aggravates CCAO-induced neuroinflammation and suggest a preconditioning effect in treated control rats, such effect was unable to prevent the cerebral damage after CCAO.

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Poster

704. Ischemia: Inflammation

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Topic: C.07. Ischemia

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Veteran's Merit Award BX000589

Title: Calcium release-activated calcium (CRAC) channel inhibition protects against experimental brain injury by inhibiting microglial activation

Authors: *R. KACIMI[1,2], J. KIM[1,2], K. STAUDERMAN[3], M. DUNN[3], S. HEBBAR[3], M. YENARI[1,2];
[1]VA Med. Center, Neurol. Dept. (N127), Univ. of California San Francisco, San Francisco, CA; [2]San Francisco VA Med. Ctr., San Francisco, CA; [3]CalciMedica Inc, La Jolla, CA

Abstract: Inflammatory responses following brain injury are known to worsen neurological outcome, and represent a potential target for therapeutic intervention. Store-operated Ca2+ entry mediated by Ca2+ release-activated Ca2+ (CRAC) channels, contributes to calcium signaling in immune cells. CRAC channels consist of endoplasmic reticulum resident Ca2+-binding/sensing protein stromal interaction molecule 1 (STIM1) and the calcium channel protein ORAI1 located in the plasma membrane. Prolonged Ca2+ entry through CRAC channels activates, via

calcineurin, nuclear factor of activated T cells (NFAT), involved in T cell proliferation and cytokine expression. Microglia mediate inflammation in the injured brain, but little is known whether CRAC channels are involved. We studied novel CRAC channel inhibitors to explore their therapeutic potential in models of microglia-mediated neuronal injury. Neuro-2A cells were cultured alone or with microglial BV2 cells, then exposed to 2h oxygen glucose deprivation (OGD). Some cultures were treated with a novel CRAC channel inhibitor, after which cell viability was determined. Toll-like receptor (TLR) -3 , -4 agonists or IFNγ were used to induce inflammatory responses in microglia. Western blots revealed the presence of the canonical CRAC channel proteins STIM1 and ORAI1 in BV2 cells. CRAC channel inhibition decreased NO release and inflammatory proteins iNOS and COX-2 expression in activated microglia. Basal cytoplasmic calcium levels in microglia were elevated by a TLR-4 agonist, and were reduced by CRAC channel inhibition. Similarly, a TLR-4 agonist activated JNK1/2 kinase, NFAT, NF-κB, CREB & STAT1, but only JNK1/2 kinase & NFAT were attenuated by the inhibitor. OGD decreased N2A neuronal cell viability, further exacerbated by BV2 cells, but cells were protected by the CRAC channel inhibitor (n=3-5, *$p<0.05$). We then treated C57/BL6 mice exposed to experimental brain trauma (TBI) and found that treatment led to decreased lesion size, brain hemorrhage and improved neurological deficits (n=6-7/grp, *$p<0.05$). CRAC channel inhibition led to neuroprotection, mediated at least in part through JNK and transcription factor NFAT signaling pathways. We suggest a novel anti-inflammatory approach for treating acute brain injury. Our observations also shed light on new calcium signaling pathways, not previously described in brain injury models.

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Poster

704. Ischemia: Inflammation

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Topic: C.07. Ischemia

Support: CIHR

Title: Impaired microglial responses to cerebral microbleeds in hyperglycemic mice can be prevented with insulin therapy.

Authors: *S. L. TAYLOR[1], E. R. WHITE[2], C. E. BROWN[2];
[1]Div. of Med. Sci., [2]Univ. of Victoria, Victoria, BC, Canada

Abstract: Approximately 7-9% of the population is living with some form of diabetes. When poorly controlled (which is often the case), this disease is associated with cerebrovascular pathology such as microbleeds and impairments in cognitive function. The presence and burden of microbleeds in the brain has been strongly linked with reduced cognitive function and increased risk of dementia. Microglia are the resident immune cells of the central nervous system, that dynamically respond to vascular insults by extending their processes to the site of injury. The rapid actions of microglia are thought to play a beneficial role in vascular repair since inhibiting these responses can exacerbate injury. Here, we hypothesized that diabetes, especially if not well controlled with insulin, will disrupt microglia based repair of damaged microvessels in the brain. Using 2-photon in vivo imaging, we show that chronic hyperglycemia in the streptozotocin model of type 1 diabetes leads to decreased microglial process accumulation around the site of microvascular injury. Importantly, this impaired microglial response could be prevented with tight control of blood glucose levels with insulin. These results indicate that chronic hyperglycemia disrupts microglial based repair of damaged microvessels, which may help explain why poorly controlled diabetes is associated with greater risk of cerebrovascular dysfunction and cognitive decline.

Disclosures: S.L. Taylor: None. **E.R. White:** None. **C.E. Brown:** None.

Poster

704. Ischemia: Inflammation

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Topic: C.07. Ischemia

Support: NIH K99NR013593 (KPD)

Title: Characterization of inflammation in acute and non-acute ischemic infarcts in human and mouse brain tissue

Authors: *T.-V. V. NGUYEN[1], J. B. FRYE[2], J. C. ZBESKO[2], A. URZUA[2], K. STEPANOVIC[2], M. HAYES[2], K. P. DOYLE[3];
[1]Departments of Immunobiology and Neurol., [2]Dept. of Immunobiology, [3]Departments of Immunobiology, Neurology, and the Arizona Ctr. on Aging, Univ. of Arizona, Tucson, AZ

Abstract: Approximately 30% of stroke survivors develop dementia following a stroke. We hypothesize that one of the mechanisms that contributes to this delayed cognitive decline is a chronic inflammatory response that persists at the site of the stroke lesion and causes bystander damage to surrounding tissue. To address this hypothesis, we present a comprehensive

characterization of the cytokine and chemokine response to stroke in the human brain at different stages of wound healing. Our data indicate that inflammation following stroke may not be as efficiently self-limiting as it is in other tissues. In addition, due to most basic and preclinical research being conducted in rodent models of stroke, we also present a comparison of the cytokine and chemokine response to stroke in human and mouse brains at two different stages of wound healing, using C57BL/6 and BALB/c mice to control for strain related differences in the immune response. Our data indicate that the acute inflammatory response to stroke is largely conserved in mice as well as in humans. In each mouse strain and in humans, there is a significant increase in levels of GM-CSF, IL-6, IL-12 (p70), IP-10, KC/IL-8, MCP-1, MIP-1α, MIP-1β, RANTES, and TNFα in the infarct core during the acute time period. However, in the weeks after stroke, during the stage of liquefactive necrosis, the chronic inflammatory response to stroke diverges in both mouse strains and humans; only the sustained elevation of IL-6 and MCP-1 is conserved in mice and humans, with each strain of mouse and each species displaying a unique profile of chronically elevated pro-inflammatory cytokines in the area of infarction. Furthermore, because nearly 75% of all strokes occur in patients over the age of 65, and approximately 25% of strokes are recurrent strokes, we also present findings that reveal how the inflammatory response to stroke is impacted by age and multiple strokes in mice. Our data indicate that the chronic inflammatory response to stroke is not exacerbated by age in 18-month old mice compared to 3-month old mice but is significantly exacerbated by a recurrent stroke.

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Poster

704. Ischemia: Inflammation

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Takeda Science Foundation

Title: Development of a novel anti-inflammatory and anti-osteoclastic peptide based on RANKL for treatment of ischemic stroke

Authors: ***H. KURINAMI**[1], M. SHIMAMURA[2], H. NAKAGAMI[3], H. MOCHIZUKI[2], R. MORISHITA[4];

[1]Osaka Univ., Suita/Osaka, Japan; [2]Dept. of Neurol., [3]Dept. of Hlth. Develop. and Med., [4]Osaka Univ. Grad. Sch. of Med., Suita, Japan

Abstract: Background & Purpose: We previously showed that stimulation of RANKL (receptor activator of nuclear factor-B ligand)/ RANK (receptor for RANKL) signal with recombinant RANKL worked as protective in ischemic brain through inhibiting TLR signaling pathways. However, augmentation of RANK signaling is also causative for increasing osteoclast differentiation and osteoporosis, which is one of risk factors in poor prognosis in ischemic stroke. To overcome the problem, we developed a novel RANKL-based peptide, which is anti-inflammatory and anti-osteoclastic.
Methods: We made three peptides: MHP1 and 2 with DE loop and EF loop; MHP3 including complete CD loop and DE loop. The action of the peptides was evaluated in MG6 cells (microglia), RAW 264.7 cells (macrophage), primary osteoclast precursor cells, and primary mixed neuron-glia cultures. The effects of the peptide were also checked in 40 min-transient cerebral artery occlusion (tMCAo) model in C57BL6/J mice.
Results: In LPS-stimulated MG6 and/or RAW 264.7 cells, MHP1 and MHP2 inhibited expression of IL-6 and TNFα, whereas MHP3 showed no effects. This anti-inflammatory effects of MHP1 was dependent on RANK signals because knockdown of RANK showed lack of action. The effects of anti-inflammation of MHP1 were also shown in LPS-stimulated primary mixed neuron-glia cultures. In primary osteoclast precursor cells, MHP1 did not stimulate osteoclast differentiation, which is quite different from recombinant RANKL. Interestingly, MHP1 also inhibited RANKL-induced osteoclast differentiation. The expression of IL-6 and TNFα was prevented in the stimulation of FSL-1 (a ligand for TLR2/6) but not S100β (a ligand for RAGE), indicating that the action of MHP1 might be specific in TLRs signaling. Finally, we injected MHP1 intracerebroventricularly at 4 hrs after the ischemia and evaluated the effects. MHP1-treated mice showed less infarct volume and activated microglia/macrophages in ischemic brain at 72 hrs after ischemia.
Conclusions: Thus, MHP1 is a partial agonist of RANKL, which could inhibit TLR-related inflammations without activation of osteoclasts. MHP1 could be a novel agent for preventing inflammation and osteoporosis in ischemic stroke.

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Poster

704. Ischemia: Inflammation

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Program#/Poster#: 704.11/AA6

Topic: C.07. Ischemia

Title: Estrogen regulation of inflammasome activation and microglial phenotype in the hippocampus after global cerebral ischemia

Authors: *R. D. THAKKAR[1], R. WANG[1], Q. ZHANG[1], R. VADLAMUDI[2], D. BRANN[1]; [1]Dept. of Neurosci. and Regenerative Med., Med. Col. of Georgia, Augusta Univ., Augusta, GA; [2]Dept. of Obstetrics and Gynecology, Univ. of Texas Hlth. Sci. Ctr., San Antonio, TX

Abstract: 17β-estradiol (E2) is a well-known neuroprotective factor in the CNS. Recently, our lab showed that the estrogen receptor (ER) co-regulator protein, Proline-, glutamic acid-, and leucine-rich protein 1 (PELP1) is critical for its neuroprotective and cognitive effects. In the current study, we examined whether E2, acting via PELP1, can exert anti-inflammatory effects in the ovariectomized rat and mouse hippocampus to regulate NLRP3 inflammasome activation, cytokine production and microglial M1/M2 phenotype after global cerebral ischemia (GCI). The results showed that activation of the NLRP3 inflammasome pathway and expression of its downstream products, cleaved caspase-1, and IL1β, are temporally increased in the hippocampus after GCI, with peak levels observed at 6-7 days. E2 robustly inhibited NLRP3 inflammasome pathway activation, caspase-1 and pro-inflammatory cytokine production, as well as gliosis after GCI at gene as well as protein levels. Moreover, E2 also profoundly suppressed the pro-inflammatory M1 microglial phenotype, while increasing the anti-inflammatory M2 microglial phenotype early after GCI. Intriguingly, the ability of E2 to exert all of these anti-inflammatory effects was lost in PELP1 forebrain-specific knockout mice. Collectively, our study demonstrates that E2 signaling via the ER co-regulator, PELP1 exerts robust anti-inflammatory effects in the hippocampus after GCI to regulate inflammasome activation, cytokine production and microglia phenotype. Thus, in addition to its neuroprotective actions, E2 can also exert robust anti-inflammatory effects following ischemic injury to the brain.

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Poster

704. Ischemia: Inflammation

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Topic: C.07. Ischemia

Title: Oosteopontin attenuates secondary neurodegeneration in the thalamus after experimental stroke

Authors: *M. SCHROETER[1], A. LADWIG[1], J. HUCKLENBROICH[2], A. WILLUWEIT[3], M. SCHOENECK[3], K.-J. LANGEN[3], G. R. FINK[1,4], M. RUEGER[1];
[1]Dept. of Neurology, Univ. Hosp. Cologne, Cologne, Germany; [2]Quiagen GmbH, Hilden, Germany; [3]INM-4, [4]Inm-3, Res. Ctr. Juelich, Juelich, Germany

Abstract: Background: Cortical cerebral ischemia elicits a neuroinflammatory reaction not only at the site of the infarction, but also in remote areas. Typically, neurodegeneration affects reciprocally connected thalamic nuclei. Thalamic injury contributes to persisting clinical disability, pain syndromes, and neuropsychiatric problems after stroke. Osteopontin (OPN) is a cytokine-like glycoprotein that is excreted in high amounts after cerebral ischemia and which exerts various immunomodulatory functions. In this study we examined its protective effects on thalamic neurons.
Methods: Male wistar rats were subjected to photothrombosis and subsequently injected intracerebroventricularily with 500µg OPN or vehicle. Immunohistochemical and fluorescence staining were used to detect inflammation (Iba1, Ox42, ED1), neurodegeneration (NeuN), regenerative processes (NG2), cell proliferation (BrdU), microglia M1 (iNOS) and M2 polarisation (CD206, Arg1, and Ym1). Autoradiography scans were used to visualize degeneration (D-Prolin) and proliferation (thymidine).
Results: Extensive secondary neurodegeneration with microglial activation and neurodegeneration in the thalamus was found in all animals with cortical ischemia. Microglial infiltration co-distributed with neurodegeneration. Treatment with osteopontin significantly decreased neurodegeneration, inflammation, and microglial proliferation and improved regenerative processes. Although microglia was classified as activated by morphological criteria, microglia did not express markers of M1 or M2 polarisation. D-Prolin uptake mirrored degeneration. Within the infarct tissue it was not significantly changed, whereas it visualized attenuated thalamic degeneration in OPN treated animals.
Conclusion: Thalamic secondary neurodegeneration can be ameliorated by pharmacological treatment with OPN. Whether this also improves clinical outcome warrants further investigation.

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Poster

704. Ischemia: Inflammation

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Topic: C.07. Ischemia

Title: Post-ischemic whole body vibration reduces inflammation in the brain of middle-aged female rats

Authors: *M. SCHATZ[1,4], N. D'ADESKY[4], P. BHATTACHARYA[4], A. P. RAVAL[4], D. W. DIETRICH[2], H. M. BRAMLETT[3,5];
[1]Neurol., Univ. of Miami, Weston, FL; [2]Neurolog. Surgery, [3]Neurol., Univ. of Miami, Miami, FL; [4]Neurol., Cerebral Vascular Dis. Res. Labs., Miami, FL; [5]VA, Bruce W. Carter, Miami, FL

Abstract: A woman's risk of stroke increases exponentially after menopause, and even a mild ischemic episode can result in increased frailty. Studies performed in laboratory animals and humans support that whole body vibration (WBV) reduces or reverses pathological remodeling of bone and lessens frailty-related physiological deterioration. Using a rodent model of stroke, we have examined whether WBV reduces inflammation and post-ischemic damage and improves motor function in middle-aged female rats. Middle-aged Sprague–Dawley female (9–11 months) rats were exposed to transient middle cerebral artery occlusion (tMCAo; 60 min) and randomly assigned to either WBV or control groups. Animals placed in the WBV (40 Hz) group underwent 30 days of WBV treatment performed twice daily for 15 min each session for 5 days each week. During the treatment period, we tested motor function using a rotarod test at the 7th, 15th and 30th day after tMCAo. Animals were sacrificed on 30th day of WBV treatment and brain tissue was harvested for histopathological and inflammasome protein analysis performed by western blotting. Western blot results demonstrated a two-fold decrease in the inflammasome proteins caspase-1, caspase recruitment domain (ASC), and interleukin-1β. The rotarod test scores from the WBV treatment group were significantly higher than the control group on day 30 ($p < 0.05$) at 10, 30 and 40 RPM speeds, suggesting a significant improvement in functional activity of the WBV group. Overall, WBV has shown promising results in decreasing inflammation and increasing functional activity after stroke in middle-aged female rats.

Disclosures: **M. Schatz:** None. **N. d'Adesky:** None. **P. Bhattacharya:** None. **A.P. Raval:** None. **D.W. Dietrich:** None. **H.M. Bramlett:** None.

Poster

704. Ischemia: Inflammation

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 704.14/AA9

Topic: C.07. Ischemia

Support: Ministry of Science and Technology NSC 102-2314-B-038 -025 -MY3

Title: The therapeutic effects of pomalidomide to attenuate inflammation <and> mitochondrial dysfunction of cerebral ischemia

Authors: *Y.-R. TSAI[1,2], C.-F. CHANG[3], J.-H. LAI[1,2], K.-Y. CHEN[1,2], J.-C. WU[3], Y.-H. CHIANG[3,1,2];
[1]The Ph.D. Program for Neural Regenerative Med., [2]Ctr. for Neurotrauma and Neuroregeneration, Taipei Med. Univ., Taipei, Taiwan; [3]Dept. of Neurosurg., Taipei Med. Univ. Hosp., Taipei, Taiwan

Abstract: Stroke is one of the top major leading causes of death and severe disability in the world. After cerebral ischemia, inflammation plays a key role in the development of permanent neurological damage. Evidences indicated that reactive oxygen species (ROS) was involved in the mechanism of post-ischemic inflammation. ROS is produced by activation of several inflammatory enzymes and then suppresses the activity of mitochondria causing further tissue damage. In this study, we investigated pomalidomide, an immunomodulatory drug, in in primary neuronal culture of H2O2-induced injury model and in middle cerebral artery occlusion (MCAO) rodent model of ischemic stroke. The effects of pomalidomide were consistent for in vitro and in vivo models. In vitro results, we found that use of low dose pomalidomide in H2O2-insulted neuronal culture significantly decreased the level of LDH release and MTT assay was provided results consistent with LDH assay. In addition, the protein level of complex III expression in low dose of pomalidomide with H2O2-insulted group was superior to H2O2-insulted group in primary neuronal culture. Also, the Apoptosis-inducing factor (AIF) expression, low dose of pomalidomide with H2O2-insulted group was distinctly lower than H2O2-insulted group. Similarly, for in vivo results, triphenyltetrazolium chloride (TTC) stain brain showing a markedly decreased infarct volume in pomalidomide group compared with the control group in MCAO model. In conclusion, our in vitro and in vivo results showed that pomalidomide exhibits neuroprotective effects and reduces cerebral ischemia/reperfusion injury. And these results suggest that pomalidomide may preserve mitochondiral function by reducing mitochondrial ROS production and also decrease apoptosis after ischemic brain damage.

Disclosures: Y. Tsai: None. **C. Chang:** None. **J. Lai:** None. **K. Chen:** None. **J. Wu:** None. **Y. Chiang:** None.

Poster

705. Pain: Trigeminal Processing

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 705.01/AA10

Topic: D.02. Somatosensation: Pain

Support: NSC 102-2311-B-002-034-MY3

NTU 105R891602

Title: Changes of forebrain activity after partial infraorbital nerve ligation: a manganese-enhanced magnetic resonance imaging <MEMRI > study

Authors: *W.-T. ZHAO[1,2], R.-F. CHEN[1,3], J.-H. CHEN[1,2,4,5], C.-T. YEN[1,3];
[2]Grad. Inst. of Biomed. Electronics and Bioinformatics, [3]Life Sci., [4]Electrical Engin., [5]Mol. Imaging Ctr., [1]Natl. Taiwan Univ., Taipei, Taiwan

Abstract: Peripheral neuropathy often leads to spontaneous pain, allodynia and hyperalgesia. However, the neuroplastic changes in the brain relate to the progression of neuropathic symptoms remain unclear. In this study, we used partial ligation of infraorbital nerve (pIONx) rat model to longitudinally follow behavioral and functional brain changes associated with the development of hypersensitivity. Female Sprague-Dawley rats were first trained to endure 26g of von frey filament stimulation in the whisker pad. Thereafter, each rat was scanned 3 times of whole brain MEMRI. The First time is baseline, the second time is on the same day after pIONx, and the third time is 15-d after pIONx. For MEMRI, $MnCl_2$ solution (20 mg/Kg) was given intraperitoneally and the rat was returned to home cage for 24 hours of Mn^{2+} accumulation. All MEMRI scans were acquired under isoflurane anesthesia. For pIONx, the rat was anesthetized with sodium pentobarbital (65 mg/ Kg, i.p.), and 1/3 to 1/2 of the left ION was isolated and ligated. The rat had 24hr free access to ibuprofen in the drinking water to reduce the surgical pain. Our within-animal results showed (1) elevated trigeminal ganglion and cortical activities 24 hours after pIONx. (2) Widespread cortical activity increases 15 days after surgery. Additionally, we compared group MEMRI according to behavioral response of the rat (i.e. pain group vs non-pain group) on day 15 after pIONx. Rats with neuropathic pain showed enhanced activity in bilateral cingulated cortex, nucleus accumbens, and caudate putamen; ipsilateral sensorimotor cortex, orbitofrontal cortex, and insular cortex. These observations suggest that partial ligation of infraorbital nerve increases limited cortical regions in 24 hours and neuropathic pain symptoms were associated with increased limbic and sensorimotor activity.

Disclosures: **W. Zhao:** None. **R. Chen:** None. **J. Chen:** None. **C. Yen:** None.

Poster

705. Pain: Trigeminal Processing

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 705.02/AA11

Topic: D.02. Somatosensation: Pain

Support: Facial Pain Research Foundation

Title: Neural correlates of trigeminal pain

Authors: *Q. ZHAO[1], C. SPECTOR[2], J. NEUBERT[2], M. DING[1];
[1]J. Crayton Pruitt Family Dept. of Biomed. Engin., [2]Dept. of Community Dent. & Behavioral Sci., Univ. of Florida, Gainesville, FL

Abstract: Trigeminal Neuralgia (TN) is a debilitating chronic facial pain disorder. It is generally believed that focal demyelination due to vascular compression of the trigeminal nerve is the cause of the condition. However, unpredictable responses to surgical treatment, and variability in long-term clinical outcomes suggest that TN may involve both peripheral and central mechanisms. The goal of this study was to investigate the neural correlates of trigeminal pain. Eight patients (6 females) clinically diagnosed with TN enrolled and underwent MRI scanning. The scanning session had two periods: (1) pain rating period and (2) visuomotor tracking period, each lasting 10 minutes. During the pain rating period, subjects rated the moment-to-moment intensity fluctuations of their facial pain using a trackball on a visually displayed 0-100 scale. During the visuomotor tracking period, subjects were provided with the pain intensity fluctuations recorded from the pain rating period and instructed to track the cursor movement with the trackball. During analysis the visuomotor tracking period was subtracted from the pain rating period to remove motor-related activations. Simultaneous EEG-fMRI was recorded from the patients during the experiment. One subject did not complete the scan and was excluded. Analysis of the remaining 7 subjects revealed the following results. First, correlating the pain intensity fluctuation with blood-oxygen-level-dependent (BOLD) activity, we found that BOLD in insula and anterior cingulate cortex (ACC) was positively correlate with pain intensity, whereas BOLD in thalamus and postcentral gyrus was negatively correlated with pain intensity. Second, extracting the power of theta-band (4 to 8 Hz) EEG oscillations from frontal channels and the power of alpha-band (8 to 12 Hz) EEG oscillations from occipital channels using a moving window, we found evidence that theta and alpha power was correlated with the pain intensity fluctuation in some patients. Third, correlating theta power with BOLD, we found positive BOLD-theta correlation in insula, and negative BOLD-theta correlation in thalamus and ACC. Fourth, correlating alpha power with BOLD, we found positive BOLD-alpha correlation in insula, ACC and fusiform face area, and negative BOLD-alpha correlation in thalamus, putamen and caudate. These results demonstrate that TN was associated with the activation and deactivation of a complex brain network. Rhythmic brain oscillations might provide an index of the level of pain intensity.

Disclosures: **Q. Zhao:** None. **C. Spector:** None. **J. Neubert:** None. **M. Ding:** None.

Poster

705. Pain: Trigeminal Processing

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Program#/Poster#: 705.03/AA12

Topic: D.02. Somatosensation: Pain

Support: Texas Norman Hackerman Advanced Research Program (003656-0071-2009)

TXMRC

NIH grant DE022129 from NIDCR

Title: Behavioral and local field potential changes in the thalamus and anterior cingulate cortex of behaving rats experiencing post herpetic neuralgia

Authors: *J. N. STRAND[1], M. RAO[2], Y. PENG[1], L. BELLINGER[2], P. KRAMER[2];
[1]Univ. of Texas At Arlington, Arlington, TX; [2]Dept. of Biomed. Sci., Baylor Col. of Dentistry, Texas A&M Univ., Dallas, TX

Abstract: Post herpetic neuralgia (PHN) is experienced as burning pain in the facial region, caused by nerve damage from the herpes zoster virus. The pain often becomes chronic and debilitating. Investigations into the pain pathway become vital. Pain itself is multidimensional, consisting of both the sensory and affective experiences. One of the primary brain substrates for transmitting sensory signals in the facial muscles is through ventral posterior medial thalamus (VPM). Anterior cingulate cortex (ACC) has been shown to be vital in the affective experience of pain, so investigating both of these areas in freely behaving animals can provide important information. Local field potential (LFP) recordings measure changes to the summation of subthreshold neuronal activity. A new investigational method of using a designer receptor activated by a designer drug (DREADD) (Armbruster et al, 2007) has been shown by our lab to inhibit both neuronal activity in the VPM and spontaneous pain behavior in a formalin pain model in rats. By application of DREADD, the LFPs of both VPM and ACC were recorded simultaneously to see how the inhibition of neuronal activity in the VPM affects neuronal activity in the ACC in freely behaving rats in a more clinically relevant pain model, PHN. Additionally, a place escape-avoidance paradigm was used to measure affective experience of pain (Fuchs et al, 2014). Methods: DREADD was infused into the VPM of Sprague-Dawley rats and LFP recording electrodes were implanted into the VPM and ACC. A week later, the whisker pads were injected with either varicella zoster virus (VZV) infected cells or MeWo (control) uninfected cells. Within 7-14 days, the LFP for both VPM and ACC were recorded, DREADD was activated via IP injection (or saline for control) of clozapine-N-oxide. A new baseline was recorded and the animals were tested using a preferred escape-avoidance paradigm. We found that, similar to the spontaneous pain behavior, escape-avoidance behavior is significantly

reduced in the drug group compared to the no drug group in PHN. Changes in LFP activity of theta, alpha, beta, and gamma wavelengths were noted in both the ACC and VPM with increased activity observed in PHN rats compared control and also between the drug and no drug group in PHN. Understanding how manipulation of thalamic activity can affect changes in both neuronal activity and pain behavior can bring us closer to identifying novel ways to relieve post herpetic neuralgia in humans.

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Poster

705. Pain: Trigeminal Processing

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Program#/Poster#: 705.04/AA13

Topic: D.02. Somatosensation: Pain

Support: NRF Grant 2012M-3A9B6055414

NRF Grant 2008-0062282

Title: Blockade of central of angiogenesis promotes antinociceptive effects in rats with trigeminal neuropathic pain

Authors: *D. K. AHN[1], J. SON[1], M. KIM[1], H. KIM[1], J. JU[1], K. YANG[1], M. PARK[2], M. LEE[3], H. KIM[1];
[1]Dentistry, Kyungpook Univ., Daegu, Korea, Republic of; [2]Kyung-Woon Univ., Gumi, Korea, Republic of; [3]Dong-Eui Univ., Pusan, Korea, Republic of

Abstract: Angiogenesis factors, Hypoxia-inducible factor 1 alpha (HIF-1α) and vascular endothelial growth factor (VEGF), stimulate the vascular function through modulation of endothelial cell functions and vascular permeability. The present study investigated a role of HIF-1α and VEGF in the development of trigeminal neuropathic pain. Sprague-Dawley male rats were anesthetized with ketamine (40 mg/kg) and xylazine (4 mg/kg). Under anesthesia, the left lower second molar was extracted, followed by the placement of a mini-dental implant to intentionally injure the inferior alveolar nerve. The blood-brain-barrier (BBB) permeability was assessed by detecting the concentrations of sodium fluorescein (NaF) in the medullary dorsal horn. Inferior alveolar nerve injury by mal-positioned dental implant produced prolonged mechanical allodynia compare to the sham group. Intracisternal infusion of VEGF antibody (250, 500 ng/ 24 hr/ 7 days) significantly attenuated mechanical allodynia produced by mal-positioned

dental implants. Intracisternal injection of ZM 306416 (100 μg), a VEGF receptor 1 inhibitor, or Vandetanib (20 μg), a VEGF receptor 2 inhibitor, produced inhibition of mechanical allodynia. Western blotting analysis reveals that inferior alveolar nerve injury produced up-regulation of both HIF-1α and VEGF expression in the medullary dorsal horn. Double immnuoflurosence data showed that VEGF receptor 2 co-localized with astrocyte cells but VEGF receptor 1 found in vessels stained positive with the BBB in the medullary dorsal horn. Intracisternal injection of PX-12 (50 μg), a HIF-1α inhibitor, produced inhibition of mechanical allodynia. In addition, inferior alveolar nerve injury increased extravasated NaF level which was blocked by intracisternal infusion of VEGF antibody. These results suggest that central HIF-1α and VEGF pathway play a critical role in the development of trigeminal neuropathic pain and blocking of HIF-1α or VEGF pathway is a new potential therapeutic target for neuropathic pain control including the orofacial area.

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Poster

705. Pain: Trigeminal Processing

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Program#/Poster#: 705.05/AA14

Topic: D.02. Somatosensation: Pain

Support: Migraine Research Foundation

NIH DA02110

Title: Toll like receptor 4 signaling contributes to compound 4880 evoked light aversion in a murine migraine model

Authors: *R. RAMACHANDRAN[1], C. SAVEEDRA[1], Z. WANG[2], N. MASCARENHAS[2], A. DINARDO[2], M. CORR[3], T. L. YAKSH[1];
[1]Dept of Anesthesiol., [2]Dept. of Medicine, Div. of Dermatol., [3]Dept. of Medicine, Div. of Rheumatology, Allergy and Immunol., UCSD, San Diego, CA

Abstract: Background: Neurogenic inflammation is considered to be a major component of migraine and the role of toll-like receptor 4 has been implicated in chronic inflammatory pain states. Compound 48/80 induced mast cell degranulation activates migraine pain pathway and we assessed the development of light aversive behavior, as a surrogate of photophobia following compound 48/80 in both male and female mice. We also determined the role of toll-like receptor

4 in mast cell degranulation induced migraine -like behavior and neuronal activation. **Methods:** Mice (male and female, C57Bl6), WT and TLR4 KO's were acclimatized for 15 min a day before the actual testing day in a test system composed of two equally sized chambers (light and dark) where animals could move freely through a small portal. On the day of testing baseline behavior was determined for 15 min prior to the injection with saline or the drug i.p. Following saline/compound 48/80 administration, animals were tested for 15 mins at different time points (15 min, 1 hr, 2 hrs and 4 hrs). Time spent in the light and dark box was assessed by the obscuration by the animal of red light LEDs. Effect of pre-treatment with sumatriptan (45 min) and TLR4 antagonist TAK-242 (3 hrs) were also analyzed in this model. Another group of WT and KO mice were sacrificed at 10 min following compound 48/80 for analyzing p-ERK activation in nucleus caudalis. **Results:** Compound 48/80 induced i) light aversive behavior that lasted for 2 hrs with a complete reversal to the 40/60 ratio by 4 hrs ii) neuronal activation (measured by p-ERK) in nucleus caudalis in males and females and pre-treatment with sumatriptan reversed these effects. Genetic knockouts and pharmacological intervention of TLR4 reversed the effects of compound 48/80 in males, but not in females. **Significance:** We demonstrate that i) compound 48/80 induced mass cell degranulation elicits light aversive behavior in mice ii) Reduction in light aversion was observed when pre-treated with specific anti-migraine drug sumatriptan iii) Importantly, the present study provides the first evidence for the involvement of TLR4's in initiating and maintaining migraine-like behaviour and neuronal activation in a murine migraine model.

Disclosures: R. Ramachandran: None. **C. Saveedra:** None. **Z. Wang:** None. **N. Mascarenhas:** None. **A. DiNardo:** None. **M. Corr:** None. **T.L. Yaksh:** None.

Poster

705. Pain: Trigeminal Processing

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Topic: D.02. Somatosensation: Pain

Support: NIH Grant DE021849

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Title: ASIC3 inhibitor APETx2 elicits a differential effect on neuropeptide expression in trigeminal afferent neurons after masseter muscle repetitive acid exposure

Authors: *J. MORRIS-WIMAN[1], C. G. WIDMER[2];
[1]Biomed. Sci., West Virginia Sch. of Osteo. Med., Lewisburg, WV; [2]Orthodontics, Univ. of Florida, Gainesville, FL

Abstract: Persistent orofacial pain conditions such as temporomandibular disorders involve pain in the temporomandibular joint and masticatory muscles with a predominance of pain in the jaw closing muscles such as the masseter. The biological basis for this persistent pain is still unclear and models that may mimic some portion of the pathophysiology of the muscle pain are lacking. Earlier we reported on a model for orofacial pain in which repetitive unilateral injections of acidic saline (pH 4) into masseter muscle elicited persistent bilateral pain that was associated with changes in chewing behavior and with significant increases in the expression of neuropeptides (substance P and CGRP), BDNF and ASIC3. The objective of this study was to determine if increases in expression of SP and CGRP could be prevented by an inhibitor (APETx2) specific to ASIC3. Methods: Female CD-1 mice were repetitively injected with either neutral saline (pH 7, n=5), or acidic saline (pH 4, n=5) into the right masseter separated by five days. Five mice were injected with 10µl of APETx2 in PBS (3µM) into the right masseter just prior to the second acidic saline injection; five mice were used as unmanipulated controls. Seven days after the second injection the mice were sacrificed, ganglia harvested, snap-frozen, and stored at -80° until cryosectioned. 12µm cryosections of right ganglia were immunostained for either SP or CGRP (Peninsula Labs) and images acquired using a Zeiss MRm digital camera and Axiovision software. Images were histogram-matched and thresholded to produce binary images to eliminate bias. The total number of ganglion cells and the number immunolabeled for CGRP or SP were counted for three sections, 150 µm apart, for each animal. Results: No significant differences were observed between controls or neutral saline-injected in the percentage of ganglion cells expressing either CGRP (25% controls; 29% neutral) or SP (25% controls; 30% neutral). A significant increase was detected between acidic saline-injected and all other groups in the percentage of ganglion cells immunolabeled for CGRP (71% acid) and SP (75% acid). Whereas no significant difference was detected between ganglia from APETx2-injected and controls or neutral saline-injected in the percentage of cells immunolabeled with CGRP (31% APETx2), a significantly increased number of ganglion cells expressing SP was detected in APETx2- injected (50%). Conclusions: The results of the study support a role for ASIC3 in the initiation and maintenance of persistent pain in our repetitive acidic saline injection model for orofacial pain.

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Poster

705. Pain: Trigeminal Processing

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Topic: D.02. Somatosensation: Pain

Support: IASP John J Bonica 2014 award

Title: Fusobacterium nucleatum enhances oral cancer-induced activation of primary afferent neurons innervating the tongue

Authors: *N. SCHEFF, R. VEERAMACHANENI, J. MACRAE, W. M. KWON, D. G. ALBERTSON, B. L. SCHMIDT;
New York Univ., New York, NY

Abstract: Oral cancer patients report severe function-related pain, induced by mediators from the cancer. We recently reported that bacterial communities associated with a patient's oral cancer differ from that of the patient's clinically normal anatomically matched contralateral site. Analysis of the cancer associated and normal microbiomes of 46 patients who had completed the University of California San Francisco Oral Cancer Pain Questionnaire revealed significant differences in abundance of bacterial taxa correlated with the patients' responses regarding function related pain. The cancers displayed reduced abundance of *Streptococcus* and increased abundance of *Fusobacterium*. We hypothesize that *Streptococcus mitis (S. mitis)* protects the oral microenvironment from carcinogenesis and cancer-associated pain, while *Fusobacterium nucleatum (F. nucleatum)*, implicated in colon carcinogenesis, promotes oral cancer and pain. To test this hypothesis, we co-cultured a human oral cancer cell line, HSC-3, with *F. nucleatum* or *S. mitis*, applied the culture supernatants to retrogradely-labeled primary afferent neurons innervating the tongue and imaged Ca^{2+} influx. Supernatant from HSC-3 cells with *F. nucleatum* or *F. nucleatum* alone evoked a significantly greater Ca^{2+} response in trigeminal neurons compared to HSC-3 supernatant alone, HSC3 + *S. mitis* or *S. mitis* alone. These observations suggest that *F. nucleatum* enhances neuronal activation by oral cancer and potentially worsens cancer pain. *In vivo* experiments are on-going using the 4-NQO (4-nitroquinoline 1-oxide) murine model of oral carcinogenesis. Male and female C57Bl/6 mice are treated with 100 µg/ml 4NQO in the drinking water for 16 weeks *ad libitum* and monitored for an additional 8 weeks. Pain is measured using an operant gnawing assay throughout treatment. The model recapitulates human sex differences, as female mice with tongue cancers or severely dysplastic lesions showed significantly greater pain compared to males. To determine whether *F. nucleatum* or *S. mitis* promotes or protects from oral cancer development and pain in this model, pain is being measured in an additional group of female mice, inoculated with $1x10^{10}$ of either bacteria. From our *in vitro* experiments, we expect that mice inoculated with *F. nucleatum* will show accelerated

cancer development and increased pain. This work was kindly supported by the IASP John J Bonica 2014 award.

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Poster

705. Pain: Trigeminal Processing

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Topic: D.02. Somatosensation: Pain

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Title: Sequential changes in rat cortical excitation during orthodontic treatment.

Authors: E. HORINUKI[1,2], *S. N. FUJITA[2,3], N. SHIMIZU[1,2], M. KOBAYASHI[2,3]; [1]Orthodontics, [3]Pharmacol., [2]Nihon Univ. Sch. of Dent., Tokyo, Japan

Abstract: Somatosensory information derived from the periodontal ligaments (PDL) plays a critical role in identifying the strength and direction of occlusal force. The orthodontic force often causes uncomfortable sensations including nociception around the tooth, and disturbs somatosensory information processing. However, it has remained unknown whether orthodontic treatment modulates higher brain functions, especially cerebrocortical activity. To address this issue, we first elucidated the cortical regions involved in sensory processing from the PDL. In vivo optical imaging was performed to identify the cortical responses evoked by electrical stimulation of the maxillary and mandibular incisor and the first molar PDL in the rat. In naive rats, electrical stimulation of the mandibular PDL initially evoked neural excitation in the rostroventral part of the primary somatosensory cortex (S1), the ventrocaudal part of the secondary somatosensory cortex (S2), and the insular oral region (IOR), whereas maxillary PDL stimulation elicited excitation only in S2/IOR rostrodorsally adjacent to the mandibular PDL-responding region. In contrast, maximum responses to mandibular and maxillary PDL stimulation were observed in S1 and S2/IOR, and these responses almost overlapped. Next, we examined the sequential changes in the cortical excitation induced by stimulation of the maxillary 1st molar PDL during experimental tooth movement (ETM). Optical imaging was performed 1, 3, and 7 days after ETM. To apply orthodontic force in the ETM models, the

maxillary incisors and the right first molar were bound with a closed coil spring. The ETM models showed facilitated cortical excitatory propagation in comparison with controls one day after ETM, however, the facilitation gradually recovered to the control level 3-7 days after ETM. These findings suggest that orthodontic force does not induce a long-lasting neuroplastic change in the somatosensory and insular cortices. The temporal profile of the facilitated cortical responses is comparable to that of pain and discomfort induced by clinical orthodontic treatments. Finally, immunohistochemical experiment was performed to examine the relationship between cortical responses and expression of inflammatory cytokines in PDL of the maxillary 1st molar. The peak amplitude of optical signals responding to PDL stimulation tended to be increased in parallel to the number of IL-1β and TNF-α immunopositive cells, suggesting that at least in part, the enhancement of cortical responses are induced by PDL inflammation.

Disclosures: **E. Horinuki:** None. **S.N. Fujita:** None. **N. Shimizu:** None. **M. Kobayashi:** None.

Poster

705. Pain: Trigeminal Processing

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Program#/Poster#: 705.09/AA18

Topic: D.02. Somatosensation: Pain

Title: The role of serotonin in peripheral trigeminal nociception

Authors: *C. GUERRERO-TORO[1,2], E. KILINC[2], A. ZAKHAROV[2,3], C. VITALE[1,2], M. GUBERT-OLIVE[1,2], R. GINIATULLIN[1,2];
[1]Neurobio., Univ. of Eastern Finland, Kuopio, Finland; [2]A. I. Virtanen Inst. for molecular sciences, Kuopio, Finland; [3]Kazan State Med. Univ., Kazan, Russian Federation

Abstract: Despite the generally accepted view that serotonin plays a key role in migraine pathology, the precise contribution of serotonin to this disorder remains largely unknown.To approach this issue, we studied, in rats, the action of serotonin (5-hydroxy-tryptamine, 5-HT) and its analogues on meningeal afferents, trigeminal neurons and satellite glial cells (SGC) using patch-clamp recordings and calcium imaging in primary cultures of trigeminal ganglia. In the whole mount meningeal preparation with preserved trigeminal innervation, we found that serotonin evoked a robust and long-lasting nociceptive firing (71% were either transient or persistent) in the peripheral terminals of meningeal afferents. Similar effect was induced by the specific 5-HT3 agonist m-chlorophenylbiguanide (m-CPBG). Thus, 5-HT has a pro-nociceptive peripheral effect mainly via the ligand gated 5-HT3 receptors. We have shown that intracellular calcium was elevated in 21% of the primary cultured neurons after the administration of 20 uM serotonin. Interestingly, the majority of these neurons also responded to capsaicin suggesting a

co-expression of TRPV1 and serotonin receptors. In the same manner, 21% of SGCs responded to serotonin. The specific 5-HT3 agonist m-CPBG increased intracellular calcium response in 26% of trigeminal neurons suggesting expression of 5-HT3 receptor type in neurons. In contrast, m-CPBG was not effective in SGC suggesting expression of metabotropic receptors in these glial cells. Our data suggest that serotonin acting via 5-HT3 receptor in the trigeminal nociceptive network provides an excitatory nociceptive drive. Serotonin also activate glial cells potentially contributing to chronization of migraine pain.

Disclosures: C. Guerrero-toro: None. **E. Kilinc:** None. **A. Zakharov:** None. **C. Vitale:** None. **M. Gubert-olive:** None. **R. Giniatullin:** None.

Poster

705. Pain: Trigeminal Processing

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Topic: D.02. Somatosensation: Pain

Support: Hungarian Brain Research Program KTIA_13_NAP-A-III/9.

EUROHEADPAIN FP7-Health 2013-Innovation 602633

Title: The effect of anandamide on the expression of CaMKIIα and TRPV1 in an animal model of migraine

Authors: *C. F. LABORC[1], G. NAGY-GRÓCZ[1,2], Z. BOHÁR[3], A. FEJES-SZABÓ[1], L. VÉCSEI[1,3], Á. PÁRDUTZ[1];
[1]Dept. of Neurol., [2]Fac. of Hlth. Sci. and Social Studies, [3]MTA-SZTE, Neurosci. Res. Group, Univ. of Szeged, Szeged, Hungary

Abstract: Migraine is one of the most prevalent primary headache disorders. The underlying mechanisms are still unclear, however the activation and sensitization of the trigeminal system is essential in the pathogenesis. A well-known human and animal model of migraine headache is the systemic administration of nitroglycerin (NTG), a nitric-oxide donor. It can induce an immediate headache in healthy subjects, and a migraine-like attack in migraineurs after several hours. NTG is able to activate and sensitize the trigeminovascular system in animal experiments. Calcium/calmodulin-dependent protein kinase II α (CaMKIIα) is present in the dorsal horn of the spinal cord. The peripheral inflammation induced central sensitization depends on the levels of CamKIIα, thus CamKIIα is pivotal in this mechanism. Transient receptor potential cation channel, subfamily V member 1 (TRPV1) is a non-selective cation channel which occurs in the

trigeminal nerve system. In chronic pain the expression of TRPV1 is increased, it might be involved in the sensitization process. The endocannabinoid anandamide (AEA) is the endogenous agonist of the TRPV1 and the cannabinoid receptors. Beside its antinociceptive effect it is able to reduce allodynia and hyperalgesia in animal models of neuropathic pain. Therefore the aims of our study were to investigate that if AEA and/or NTG are capable of changing the CaMKIIα, TRPV1 expression levels after systemic NTG treatment in the dorsal horn of the cervical 1-cervical 2 (C_1-C_2) segments of the spinal cord, where the secondary trigeminal neurons are located. To achieve this, we used adult, male SPRD rats (n=20). In the first group we administered physiological saline intraperitoneally (i.p.), the second group received i.p. NTG. 30 minutes prior to the NTG/saline injection and 1 hour after, we gave i.p. AEA to the animals in the third and fourth group. Four hours after the saline/NTG treatment the rats were perfused and the C_1-C_2 spinal cord was removed. CaMKIIα, TRPV1 expression levels were detected with Western-blot analysis. Our results showed that both NTG and AEA alone was able to increase CamKIIα expression in the C_1-C_2 segments. NTG and AEA together had an opposite effect on this marker, possibly via negative feedback. In the case of TRPV1, NTG increased the expression, while this response was not observed with pretreatment with AEA. Consequently AEA can prevent the NTG induced sensitization in the secondary trigeminal sensory neurons.

Disclosures: C.F. Laborc: None. **G. Nagy-Grócz:** None. **Z. Bohár:** None. **A. Fejes-Szabó:** None. **L. Vécsei:** None. **Á. Párdutz:** None.

Poster

705. Pain: Trigeminal Processing

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Program#/Poster#: 705.11/BB2

Topic: D.02. Somatosensation: Pain

Support: NRF Grant 2015R1A1A1A050275

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Title: Heterogeneous expression of purinergic receptor subtypes in rat odontoblasts

Authors: *G. CHUNG[1], B.-M. LEE[1], Y. KIM[1], C.-K. PARK[2], S. JUNG[3], S. OH[1]; [1]Oral Physiol. & Neurobio., Seoul Natl. Univ., Seoul, Korea, Republic of; [2]Gachon Univ., Incheon, Korea, Republic of; [3]Hanyang Univ., Seoul, Korea, Republic of

Abstract: Odontoblasts form dentin at the outermost surface of tooth pulp. Increasing number of evidences in recent years along with the locational advantage support the secondary role of odontoblasts as sensory or immune cells. Extracellular ATP is well characterized signaling molecule in both neuronal and immune systems, and its potential involvement in inter-odontoblast communications was demonstrated recently. In an effort to elaborate the purinergic signaling mechanism in odontoblasts, current study investigated expression of calcium-signaling ATP receptors in odontoblasts isolated from incisal teeth of 8-10 week old rats through single cell RT-PCR, and their response to ATP application in vitro by calcium imaging expreriment. While RT-PCR analysis of tooth pulp detected all seven $P2X_1$- $P2X_7$ subtypes, single cell RT-PCR analysis of acutely isolated rat odontoblasts revealed $P2X_2$, $P2X_4$, $P2X_6$, $P2X_7$, $P2Y_2$ and $P2Y_4$ expression in 23-47% of cells tested, with no evidence for $P2X_1$, $P2X_3$ and $P2X_5$ expression. Increase of intracellular Ca^{2+} concentration in response to 100μM ATP, which was repeated after pretreatment of thapsigargin or under 0 Ca^{2+} condition, suggested function of both ionotropic and metabotropic ATP receptors in odontoblasts. Positive calcium response to 2',3'-O-(benzoyl-4-benzoyl)-ATP (BzATP) and negative response to α,β-methylene ATP confirmed $P2X_2$, $P2X_4$ and $P2X_7$ as functional subunits in rat odontoblasts. The enhancement of ATP-induced calcium response by ivermectin and inhibition by 5-(3-Bromophenyl)-1,3-dihydro-2H-benzofuro[3,2-e]-1,4-diazepin-2-one (5-BDBD) further confirmed functional P2X4 subtype in odontoblasts. The functional expression of $P2X_4$ and $P2X_7$ was corroborated with scRT-PCR analysis of the cells harvested after positive response from the calcium imaging experiment. Overall, this study demonstrates heterogeneous expression of calcium-related purinergic receptor subtypes in subsets of individual odontoblasts, suggesting extracellular ATP as potential signal mediators for odontoblastic functions.

Disclosures: **G. Chung:** None. **B. Lee:** None. **Y. Kim:** None. **C. Park:** None. **S. Jung:** None. **S. Oh:** None.

Poster

705. Pain: Trigeminal Processing

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Topic: D.02. Somatosensation: Pain

Support: Brown Institute for Brain Science

Association for Migraine Disorders

NS088453

NS055251

Title: Nociceptive inputs to trigeminal nucleus caudalis neurons - implications for migraine

Authors: *B. PRADIER[1], D.-S. KIM[2], D. LIPSCOMBE[3], J. A. KAUER[1];
[1]Dept. of Mol. Pharmacology, Physiol. and Biotech., Brown Univ., Providence, RI; [2]Dept. of Anat., SoonChunHyang Univ., Asan, Korea, Democratic People's Republic of; [3]Dept. of Neurosci., Providence, RI

Abstract: Migraine is a disabling and episodic brain disorder with high prevalence and complex pathophysiology. Animal models suggest that sensitization of the trigeminal pathway plays a major role in the pathology of migraine, yet little is known about long-term changes in trigeminal afferents or their synapses in the brainstem trigeminal nucleus *pars caudalis* (TNC). We used mice expressing channelrhodopsin-YFP in TRPV1 lineage neurons (generated from TRPV1-Cre (B6.129-Trpv1tm1(cre)Bbm/J)) to investigate different forms of synaptic plasticity at nociceptive primary afferents projecting onto second order relay neurons within the TNC. We found with immuno-labeling that these afferents mostly colocalize with CGRP-containing C- and Aδ-fibers, thereby indicating that light stimulation would only activate a specific subset of primary afferents predominantly designated to the transmission of nociception. Light stimulation at the slice edge (473 nm, 0.4 - 1 msec) evoked excitatory postsynaptic currents (EPSCs) and often polysynaptic activity in neurons in laminae I-II in acutely prepared transverse TNC slices. Using low-frequency stimulation with light (LFS, 1 Hz) we robustly induced long-term depression of light-evoked EPSCs ($n = 6$, $58\% \pm 13\%$, $p < 0.05$). Similar effects were observed on light-evoked EPSCs by bath applying PACAP, a neuropeptide that induces migraine in humans and sensitizes the trigeminal pathway in mice ($n = 5$, $66\% \pm 8\%$ at 10 - 20 min after washout, $p < 0.05$). We hypothesize that reduced excitatory input onto inhibitory neurons could disinhibit projecting neurons, thereby yielding an increased net output to connected brain regions. In contrast to LFS using light, after application of electric high-frequency stimulation (HFS) EPSCs either potentiated ($n = 5$ out of 14, $\Delta > 20\%$ 20 min post stimulation) or depressed ($n = 7$ out of 14, $\Delta < 20\%$ 20 min post stimulation), while the overall average remained unaffected ($101\% \pm 14\%$). The variability in these responses may reflect the heterogeneity of the neuronal population and might differ in GABAergic vs glutamatergic postsynaptic cells. Our data demonstrate various forms of persistent synaptic plasticity, which might be relevant to migraine that depend on the type and frequency of stimulation.

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Poster

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Topic: D.02. Somatosensation: Pain

Support: KAKEN 26462824

Miyata Research Grant in A

Title: Involvement of GABAergic transmission in the sensory processing in the agranular insular cortex.

Authors: *K. ADACHI[1], M. KOBAYASHI[2], H. SAKAGAMI[1];
[1]Meikai Univ. Sch. of Dent., Sakado-Shi, Japan; [2]Pharmacol., Nihon Univ. Schoo of Dent., Tokyo, Japan

Abstract: The insular cortex (IC) has morphologically and functionally unique features compared with other sensory areas including somatosensory and visual cortices. Cytoarchitectural difference based on the pattern of granular cell layer (layer IV) divides IC into three subregions: granular (GI), dysgranular (DI) and agranular IC (AI). These subregions are connected each other, and also reciprocally connected between limbic system and thalamus. These morphological features are thought to be important for process of multimodal sensory information including gustation, olfaction, visceral and thermal sensation, and nociception in IC. Recently the voltage-sensitive dye imaging revealed that the peripheral (e.g., tongue) stimulation induced region specific excitatory responses in the middle region (approximately 1 mm rostral from middle cerebral artery) of AI. And the involvement of layers II/III pyramidal neurons in the middle region of AI with these responses was confirmed by *in vivo* whole-cell patch clamp recording. The amplitude of tongue stimuli-induuced evoked excitatory postsynaptic potentials (eEPSPs) was dependent on both the stimulation intensity and the membrane potential oscillation of AI pyramidal neurons. In this study, we investigated the effects of GABA application on the alteration of membrane potential oscillation in AI II/III pyramidal neurons. We performed *in vivo* whole-cell patch clamp recording from middle region of AI. To reconstruct morphological feature of recorded cells, only one
AI neuron was recorded in each animal. AI pyramidal neurons exhibited spontaneous membrane oscillation similar with pyramidal neurons in GI and DI (Adachi et al., 2013). Electrical stimulations (7 V) of caudal IC (around middle cerebral artery) elicited eEPSPs (11.5 ± 2.9 mV, n = 8). Repetitive electrical stimulation with 5 pulses at 50 Hz induced summation of eEPSP. Topical application of pentobarbital (2.2 nM in ACSF) reduced the incidence of up state (93.9 %) and spontaneous action potentials (42.3 %). These findings suggest that GABAergic transmission in the middle AI may be involved in sensory processes from tongue.

Disclosures: **K. Adachi:** None. **M. Kobayashi:** None. **H. Sakagami:** None.

Poster

705. Pain: Trigeminal Processing

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Topic: D.02. Somatosensation: Pain

Support: MSIP,2008-0062282

Title: Ultrastructural analysis of the synaptic connectivity of transient receptor potential ankyrin 1 (TRPA 1)-expressing primary afferent terminals in the rat trigeminal sensory nuclei

Authors: ***Y. BAE**[1], Y. CHO[1], H. HAN[1], Y. KIM[1], J. BAE[1], K. NOGUCHI[2], W. MAH[1]; [1]Sch. of Dentistry, Kyungpook Natl. Univ., Daegu, Korea, Republic of; [2]Dept. Anat. and Neurosci., Hyogo Col. of Med., Hyogo, Japan

Abstract: Transient receptor potential ankyrin 1 (TRPA 1), responding to noxious cold (<17 ℃) and pungent compounds, is implicated in the mediation of nociception, but, little is known about the processing of TRPA1-mediated craniofacial nociceptive information at the 1st relay nucleus of the CNS. To address this issue, we investigated synaptic connectivity of TRPA1-positive (+) axon terminals in the rat trigeminal sensory nuclei (TSN) by using electron microscopic immunohistochemistry and analysis of serial sections. Most TRPA1+ terminals made synaptic contact with small and medium caliber dendrites or spines. A few of them received axoaxonic synapse with pleomorphic vesicles containing endings (p-ending), which were immunolabeled for glutamic acid decarboxylase, the synthetizing enzyme for the inhibitory neurotransmitter γ-aminobutyric acid (GABA). TRPA1+ terminals showed distinct synaptic connectivities among each subnuclei of the TSN. i.e., trigeminal principal (Vp), oral (Vo) and caudal (Vc) nucleus. Number of postsynaptic dendrites per TRPA1+ terminal was significantly higher in the Vp and Vc than in Vo. But, frequency of axoaxonic synapse per TRPA1+ terminal was significantly higher in the Vp than Vo and Vc. TRPA1+ terminals showing synaptic contact with soma or primary dendrites were frequently observed in the Vo but were rare in the Vp and Vc. These findings suggest that TRPA1-mediated nociception is processed in a distinct manner among each subnuclei of the TSN which is associated with distinguished neural circuits.

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Poster

705. Pain: Trigeminal Processing

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Topic: D.02. Somatosensation: Pain

Support: NIH Grant R01NS078263

Title: Cortical spreading depression evokes mechanical sensitization of meningeal nociceptors mediated by astrocyte signaling.

Authors: *J. ZHAO, D. LEVY;
Beth Israel Deaconess Med. Ctr., Boston, MA

Abstract: We have shown previously that cortical spreading depression (CSD), the underlying cause of the migraine aura, promotes persistent activation of trigeminal meningeal nociceptors - a likely source of the migrainous intracranial headache. Here we report that in rats, CSD also leads to increased mechanical responsiveness of meningeal nociceptors, a mechanism suggested to mediate the headache exacerbation during activities that involve increased intracranial pressure and brain motion. In-vivo extracellular recording of the afferents' responses to threshold and suprathreshold mechanical stimulation of the meninges revealed increased mechanosensitivity in about 50% of the units tested. Sensitization was detected in most cases at 15 min following the CSD and was maintained for at least 30 min. Local treatment with the astrocyte toxins L-α-Aminoadipic acid or sodium fluoroacetate blocked CSD-evoked sensitization of meningeal nociceptors. Local administration of carbenoxolone, which blocks connexin and pannexin gap junctions that are involved in astrocyte signaling also inhibited the CSD-evoked mechanosensitization. Local treatment with probenecid, which only inhibits pannexins, did not block the CSD-evoked sensitization. ATP, which is released during CSD potentially through connexin hemi-channels, could mediate the sensitization. Local treatment with PPADS, a broad-spectrum antagonist of the P2X ATP receptor family inhibited CSD induced sensitization. However, treatment with TNP-ATP, a more selective inhibitor of $P2X_3$ and $P2X_{2/3}$ ATP receptors, which are expressed by nociceptors, was ineffective. None of the inhibitors tested had an effect on the CSD-evoked persistent activation of meningeal nociceptors. Our data suggest that CSD-evoked meningeal nociceptor sensitization is mediated by cortical astrocytes and involves connexin and ATP signaling. Activation of nociceptive $P2X_3$ and $P2X_{2/3}$ receptors is unlikely to mediate the sensitization. However, upstream ATP signaling, potentially mediated through astrocytic P2X7 receptors, could play a role. CSD induced sensitization and activation of meningeal nociceptors likely involve different signaling pathways.

Disclosures: J. Zhao: None. **D. Levy:** None.

Poster

706. Pain Models: Human Studies

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Topic: D.02. Somatosensation: Pain

Support: Else Kröner-Fresenius-Stiftung

Title: Chronic back pain patients learn to control spinal nociception under feedback about their nociceptive withdrawal reflex (RIII reflex).

Authors: *S. KRAFFT[1,2], H.-D. GÖHMANN[3], J. SOMMER[4], A. STRAUBE[1], R. RUSCHEWEYH[1];
[1]Dept. of Neurol., [2]Grad. Sch. of Systemic Neurosciences, Ludwig-Maximilians-University Munich, Munich, Germany; [3]Dept. of Anesthesia, Intensive Care and Pain Therapy, Hosp. of Traunstein, Traunstein, Germany; [4]Dept. of Psychiatry and Psychotherapy, Philipps-University Marburg, Marburg, Germany

Abstract: Question: The descending pain inhibition is a powerful endogenous pain control system, originating in the brainstem, inhibiting nociceptive transmission in the spinal dorsal horn, and therefore reducing nociceptive input to the brain. The descending inhibition is susceptible to cognitive-emotional modulation. The nociceptive withdrawal (RIII) reflex is a measure of spinal nociception. Young healthy subjects can learn to use cognitive-emotional strategies to suppress their spinal nociception when receiving feedback about their RIII reflex, likely by learning to deliberately activate their descending pain inhibition. Here, we investigated if RIII feedback training also improves descending pain inhibition in chronic pain patients and may help to reduce clinical pain.
Methods: The RIII reflex was evoked every 8-12 s by electrical stimulation of the sural nerve and recorded from the biceps femoris muscle for 8 min. During min 4 to 6, subjects used self-selected cognitive-emotional strategies to reduce the RIII reflex, while receiving immediate feedback about their RIII reflex size. Subjects were: Chronic back pain patients receiving 1) true (n = 18) or 2) sham RIII-feedback (FB, n = 15), and 3) healthy controls receiving true RIII-feedback (n = 15). Before and after the three training sessions, we assessed descending pain inhibition using the Conditioned Pain Modulation (CPM) paradigm and clinical back pain intensity on the numerical rating scale [0-10].
Results: All three groups achieved a significant RIII-suppression, to 82 ± 13% (true-FB patients), 89 ± 14% (sham-FB patients) and 76 ± 26% (true-FB controls, all groups $p \leq 0.05$) of baseline, with simultaneous electrical pain reduction (all groups $p < 0.001$). RIII-suppression was significantly smaller in sham-FB patients than in true-FB controls ($p < 0.05$). After training, only true-FB patients had significantly improved their CPM effect (by 18 ± 23%, after vs. before

training: $p < 0.01$) and showed a significant reduction of average (-0.8 ± 1.5 [0-10]; $p < 0.05$) and maximum (-1.4 ± 1.8; $p < 0.01$) back pain. Sham-FB patients neither showed a significant improvement of CPM (by 6 ± 28%, $p = 0.4$) nor a reduction of back pain (-0.3 ± 1.1, $p > 0.2$). Discussion: Results suggest that chronic pain patients are able to learn control over their spinal nociception under feedback of the RIII reflex, to a somewhat lesser extent than healthy subjects. Furthermore, RIII feedback training improved the descending pain inhibition (CPM) and chronic back pain in chronic pain patients. Consequently, RIII feedback training might be a useful method to enhance endogenous pain inhibition in chronic pain patients.

Disclosures: S. Krafft: None. **H. Göhmann:** None. **J. Sommer:** None. **A. Straube:** None. **R. Ruscheweyh:** None.

Poster

706. Pain Models: Human Studies

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Topic: G.03. Emotion

Support: ERC Starting Grant #313000

Academy of Finland MIND program grant 265915

Title: Availability of cerebral μ-opioid and type-2 dopamine receptors predicts haemodynamic responses to vicarious pain: A combined fMRI and PET study

Authors: T. KARJALAINEN[1,2], J. LAHNAKOSKI[1], E. GLEREAN[1], H. KARLSSON[2], P. NUUTILA[2], I. P. JÄÄSKELÄINEN[1], M. SAMS[1], R. HARI[1], *L. NUMMENMAA[1];
[1]Aalto Univ., Espoo, Finland; [2]Turku PET Ctr., Turku, Finland

Abstract: Background Functional neuroimaging experiments suggest that seeing others in pain activates a part of the brain circuitry involved in first-hand experience of pain. Accordingly, it has been proposed that observers automatically mimic the affective state of the individual they see suffering from pain, which possibly promotes prosociality and helping behaviour. It has been speculated that endogenous mu-opioid (MOR) and dopamine D_2 receptor (D2R) systems, the primary neurotransmitter systems modulating physical pain, would also underlie vicarious pain. Here we search for the first direct *in vivo* evidence for this proposal.
Methods We studied 34 healthy subjects with positron emission tomography (PET) to quantify MOR and D2R availabilities, and, subsequently, with functional magnetic resonance imaging (fMRI) to unravel haemodynamic brain activity elicited by movie clips (total duration 20 min)

containing painful and painless scenes. We then explored whether regional MOR (anterior cingulate cortex, thalamus, insula) and D2R availabilities (striatum) would explain individual differences in haemodynamic responses to vicarious pain.

Results Seeing others in pain increased fMRI signals in cortical and subcortical areas known to be involved in perception of physical pain, including anterior insular cortex, thalamus and secondary somatosensory cortex. MOR availability in the regions specified above correlated *negatively* with haemodynamic responses in various parts of the pain matrix, including anterior insula, anterior cingulate cortex (ACC), thalamus, and the primary and secondary somatosensory cortices. On the contrary, striatal D2R availability correlated *positively* with haemodynamic activity in multiple brain regions, including ACC, cerebellum and primary somatosensory cortex.

Conclusions Because both opioid and dopamine systems mediated haemodynamic activity to vicarious pain, our results are in line with the idea that partially shared neurochemical pathways support first-hand physical and vicarious pain.

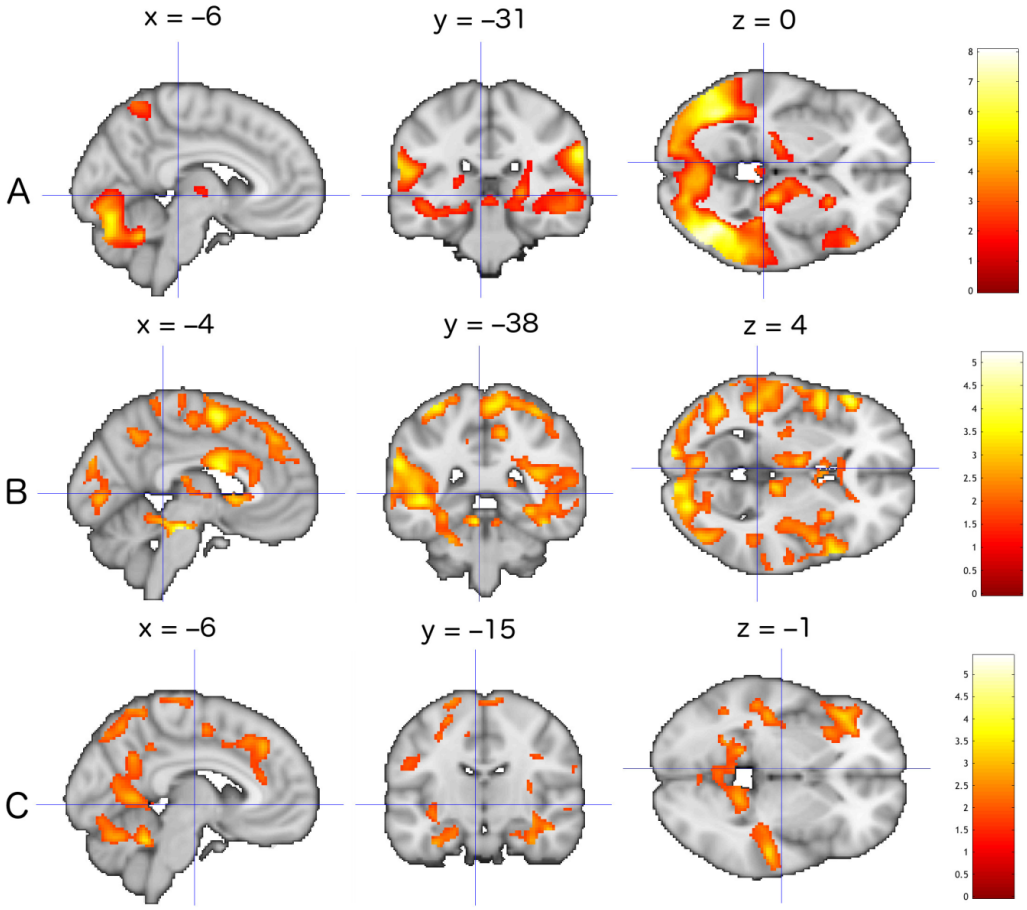

Figure 1. (A) Brain regions responding to vicarious pain during BOLD-fMRI. (B) Brain regions whose BOLD responses to vicarious pain depended negatively on MOR availability in the insula. (C) Brain regions whose BOLD responses to vicarious pain depended positively on D2R availability in the putamen. All data are thresholded at $p < 0.05$, FDR corrected at the cluster level.

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Poster

706. Pain Models: Human Studies

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Topic: D.02. Somatosensation: Pain

Support: Fondation chiropratique du Québec

NSERC of Canada

Université du Québec à Trois-Rivières

Title: Integrity of endogenous pain inhibition mechanisms in patients with chronic non-specific low back pain

Authors: ***A. LADOUCEUR**[1], J.-D. DUBOIS[2], N. RUSTAMOV[3], A. LEHMANN[4], M. DESCARREAUX[5], P. RAINVILLE[6], M. PICHÉ[3];
[1]Univ. Du Québec À Trois-Rivières, Montréal, QC, Canada; [2]Dept. of psychology, [3]Dept. of chiropractic, Univ. Du Québec À Trois-Rivières, Trois-Rivières, QC, Canada; [4]Dept. of otolaryngology, McGill university, Montreal, QC, Canada; [5]Univ. du Québec à Trois-Riviéres, Department of Humans Kinetics, QC, Canada; [6]Dept. of stomatology, Univ. de Montréal, Montreal, QC, Canada

Abstract: Chronic non-specific low back pain (LBP) affects a large proportion of the population and its pathophysiology remains undefined. As for other chronic pain conditions, one of the mechanisms potentially contributing to chronic non-specific LBP is the alteration of pain inhibition processes, including conditioned pain modulation (CPM) and pain inhibition by selective attention. Thus, the aim of this study was to determine whether CPM and pain inhibition by selective attention are altered in patients with chronic non-specific LBP. Seventeen participants with chronic non-specific LBP and age/sex matched controls were recruited. CPM and pain inhibition by selective attention were assessed while controlling for non-specific temporal effects. The experimental design comprised 3 sessions including 4 blocks: 1) painful test stimulus (baseline) 2) painful test stimulus with heterotopic innocuous cold (innocuous conditioning) 3) painful test stimulus with heterotopic noxious cold (noxious conditioning) 4) painful test stimulus (recovery). Selective attention was manipulated across two of these sessions

by instructing participants to focus their attention on either the test or the conditioning stimulus. In the third session (control), the same test stimulus was delivered in 4 blocks without any conditioning or attention intervention. The painful test stimulus consisted in transcutaneous electrical stimulation of the sural nerve while innocuous and noxious cold stimuli (conditioning) consisted in contact cold and a frozen ice pack applied on the left upper limb, respectively. Pain inhibition was not significantly different between groups across sessions and blocks ($p = 0.99$). However, it was different between sessions across blocks when combining all participants from both groups ($p < 0.001$). Indeed, pain inhibition was significantly greater when attention was focussed on the innocuous conditioning stimulus in comparison to when attention was focussed on the test stimulus ($p = 0.008$). This indicates pain inhibition by selective attention. For CPM assessed with attention focussed on the test stimulus, noxious conditioning produced greater pain inhibition compared with innocuous conditioning and this effect was greater compared with the corresponding contrast (block 3 vs 2) in the control session ($p = 0.049$). This shows significant CPM. Altogether, these results suggest that patients with chronic non-specific LBP have similar CPM and pain inhibition by selective attention compared with age/sex matched controls. Therefore, chronic non-specific LBP seems to rely on mechanisms other than altered pain inhibition processes assessed in this study.

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Poster

706. Pain Models: Human Studies

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Topic: D.02. Somatosensation: Pain

Support: NIH-NIA Grant RO1AG039659

NIH Grant 1T32AG049673

Title: Testing assumptions in human pain models: Psychophysical differences between first and second pain.

Authors: ***N. R. ECKERT**, C. J. VIERCK, C. B. SIMON, Y. CRUZ-ALMEDIA, R. FILLINGIM, J. L. RILEY, III;
Univ. of Florida, Gainesville, FL

Abstract: Acute pain arises from activation of myelinated (A delta) and unmyelinated (C) nociceptive afferents, leading to 1st (A-fiber) or 2nd(C-fiber) pain sensations. The current study sought to investigate first and second pain within glabrous and hairy skin sites in human upper limbs.Fifty healthy adults (25 males/25 females, 18-30 yrs: mean = 20.5+- 1.4) participated in a psychophysical study investigating electronically rated, thermal first and second pain sensations within the glabrous skin at the palm and hairy skin of the forearm. Repeated measures ANOVA indicated that the threshold for first pain was lower(more sensitive) than for second pain ($p = .004$), and thresholds at glabrous skin were higher than for hairy skin ($p = .001$). Hairy skin presented a steeper slope for testing, whereas there were no differences in slope between first and second pain. The study findings support assumptions associated with mechanistic differences between first and second pain sensations, while offering a novel method for producing first and second pain with the same thermal stimulus. Efforts to understand abnormalities among people with clinical pain and development of new therapeutic agents will benefit from specific psychophysical methods.

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Poster

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Topic: D.02. Somatosensation: Pain

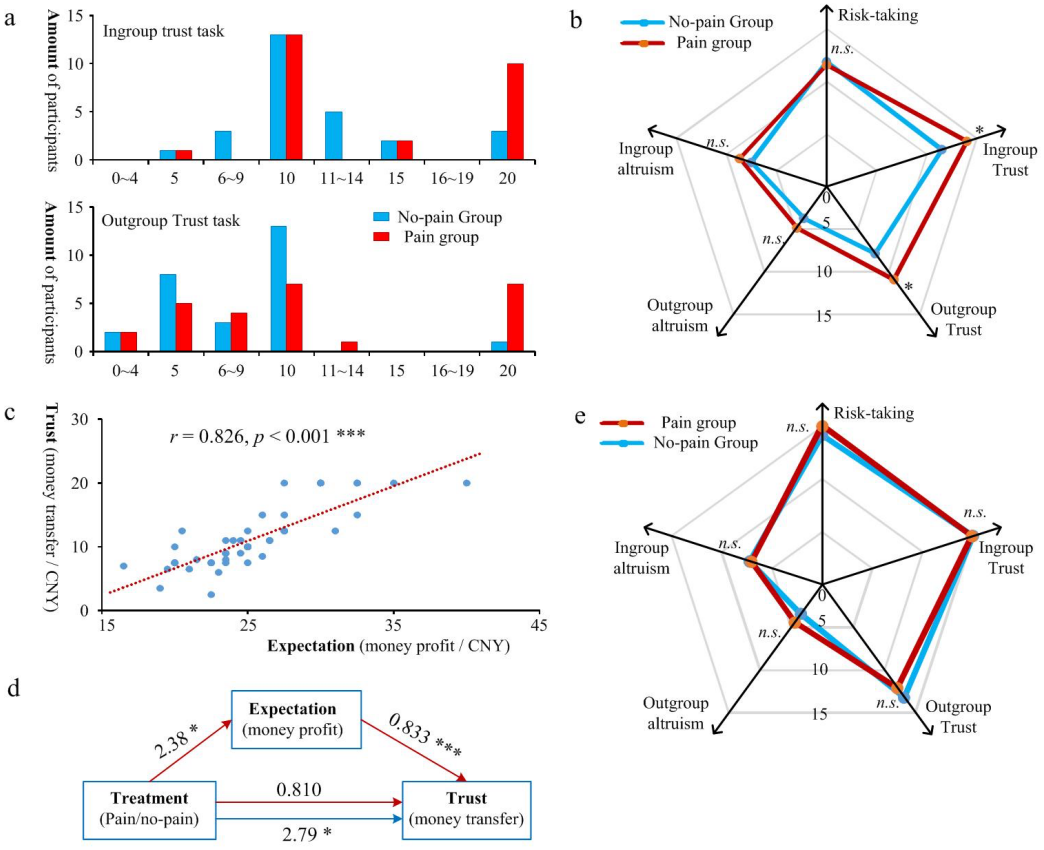
Support: China Postdoctoral Science Foundation (2015M571516)

Title: Females in physical pain trust more on others for self-interest

Authors: *C. WANG, J. GAO, X. DONG;
East China Normal Univ., Shanghai City, China

Abstract: Pain modulates cognition and brain activities in humans. Research found that pain enhances interoception sense and self-focus; meanwhile, individuals in pain are motivated to reconcile the painful feeling by pursuing potential reward (Navratilova & Porreca, 2014). As a consequence, social interactions might be modulated among painful individuals. However, it's not clear about the mechanisms of how pain influences social cognition and behaviors. To address the issue, 112 healthy university students were recruited. They were treated with either Capzasin cream (pain group) or hand cream (control group) and manipulated with intergroup relationship. Subsequently, participants completed the trust game, the dictator game and the

investment game (Cameron et al., 2013), by which levels of trust, altruism and risk taking were measured. Results showed that female subjects in acute pain (compared with control condition) sent more money transfers (12.5 vs. 9.7, p = 0.024) to intergroup members (Fig. a), which indicated that physical pain increased trust. In contrast, female participants showed no difference on altruism and risk-taking behaviors (Fig. b). In addition, the trust level was positively correlated with individual's expectation of money profit (Fig. c). Furthermore, mediation analysis revealed that the effect of pain on trust was mediated by expectation of money profit, which was confirmed by bootstrap analysis (Effect Size = 1.98, CI = [0.019 3.00], Fig. d). We further tested the pain effects on male sample and the whole sample. Unlike females, the trusting behavior along with altruism and risk-taking behaviors of males were not affected by acute pain (Fig. e). Our findings demonstrated an effect of physical pain on trust in females, and uncovered the underlying mechanisms that increased trust by pain may not be driven by altruistic motivation, but possibly by expectation of self-interest. The study extends the biopsychosocial model of pain (Gatchel et al., 2007) and may provide rationale for improving social interactions for pain patients.



Disclosures: **C. Wang:** None. **J. Gao:** None. **X. Dong:** None.

Poster

706. Pain Models: Human Studies

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 706.06/BB12

Topic: D.02. Somatosensation: Pain

Title: Body ownership, illusory limb movement and pain perception: a virtual reality study

Authors: *M. MARTINI, B. CAOLA, A. G. LEADBETTER;
Univ. of East London, London, United Kingdom

Abstract: Recent studies have shown a crucial relationship between the motor system and pain management. For instance, transcranial direct current stimulation (tDCS) and transcranial magnetic stimulation (TMS) of the primary motor cortex, brought about a successful pain modulation in different neuropathic syndromes. Recently it has been shown that even the observation of hand movements led to an increased pressure pain threshold specific for the limb involved in the movement (left/right), as well as to a decrease in intracortical inhibition in healthy subjects. Noteworthily, the vision of the own body part in pain has been shown to be analgesic, and such effect holds true even during the vision of an avatar's body that is felt as own body. However, it is still unknown whether the vision of the movement of an embodied avatar's arm may add up to the beneficial effects of seeing limb movements. Therefore, in this study twenty-one healthy right-handed subjects were exposed to four virtual reality (VR) observational conditions, while receiving increasing ramps of heat stimulation on their right arm. After each observational conditions participants were asked to answer questions about ownership, illusory movement, attention and illusory ownership of multiple limbs, via a likert scale (1 to 7). For the VR exposure two factors were taken into account: the vision of the right avatar's arm VS a non corporeal object, both seen from a first person perspective (factor "Body"), and the movement VS stillness of the virtual arm/tube ("Movement"). Preliminary results on pain threshold show only a trend to significance of the main factor "Movement" and the interaction "Movement*Body". As expected, the conditions were a movement was involved were generally more distracting, and the conditions were the avatar's arm were shown were those were a higher significant level of body ownership was reported. The illusion of having more than one right arm was stronger in the conditions were the avatar's arm was shown. A compelling significant negative correlation has been found between the illusion of having more than one arm and the pain threshold. These preliminary results show that seeing limb movements in a body ownership set-up do not lead to a higher heat pain threshold. Moreover, as suggested in the literature, our data show that a distortion in the representation of one's own body could contribute to hyperalgesic processes to kick in.

Disclosures: M. Martini: None. **B. Caola:** None. **A.G. Leadbetter:** None.

Poster

706. Pain Models: Human Studies

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 706.07/BB13

Topic: D.02. Somatosensation: Pain

Title: The placebo analgesic effect in healthy individuals and patients: a meta-analysis

Authors: *J. T. FORSBERG[1], M. MARTINUSSEN[2], M. FLATEN[3];
[1]The Arctic Univ. of Norway, Uit, 9037, Norway; [2]The Arctic Univ. of Norway, Uit, Tromso, Norway; [3]Norwegian Univ. of Sci. and Technol., Trondheim, Norway

Abstract: Objectives: The present meta-analysis investigates whether the magnitude of placebo analgesia is different in patients compared to healthy individuals, and whether placebo analgesia is different in experimentally induced pain compared to clinical pain in patients. **Methods:** A literature search in Web of Science (ISI) on the term "placebo analgesia" was conducted. The search resulted in 68 studies, including 3924 participants. Fifty-two studies included healthy individuals and 16 studies included patients. Of the 16 studies with patients, five studies investigated clinical pain and 11 studies investigated experimentally induced pain. **Results:** The average effect size was g = 1.25 for healthy individuals and g = 1.51 for patients (n.s.). In the studies with patients, the average effect sizes of placebo treatment was g = 1.73 for experimentally induced pain and g = 1.05 for clinical pain (n.s.). However, a chi-square test revealed that there were relatively more studies in which there was a clinically significant reduction in pain with patients compared to healthy volunteers (p =.04). **Conclusions:** The findings suggest that studies on healthy individuals may underestimate the magnitude of the placebo analgesic effect in patients, and that clinical pain respond to placebo to the same degree as experimentally induced pain.

Disclosures: J.T. Forsberg: None. **M. Martinussen:** None. **M. Flaten:** None.

Poster

706. Pain Models: Human Studies

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 706.08/BB14

Topic: D.02. Somatosensation: Pain

Support: National Comprehensive Cancer Network

Celgene Corporation

Title: Minimal neuropathy with intraperitoneal nab-paclitaxel in patients with advanced malignancies

Authors: ***N. PRAKASH**[1], C. MIHAELA[2], P. FRANKEL[2], H. OPENSHAW[1], R. MORGAN[2]; [1]Neurol., [2]City of Hope, Duarte, CA

Abstract: Purpose: Previous clinical trials have demonstrated an advantage to IP chemotherapy. However neurotoxicity in the form of painful sensory neuropathy is a common treatment-limiting toxicity. A phase I study was undertaken to evaluate the safety, tolerability, and pharmacokinetics of IP *nab*-paclitaxel.
Methods: Eligible patients received IP *nab*-paclitaxel on days 1, 8, 15 of a 28-day cycle with a 3+3 dose-escalation design. Plasma and peritoneal pharmacokinetic samples were drawn prior to dose, immediately upon completion of infusion, 1, 2, 4, 6, 8, 12, 24 and 48 hours post-dose on C1D1 and C1D15. Patients also underwent voluntary neurological exam, nerve conduction studies, and quantitative sensory nerve testing at baseline and at pre-cycle three.
Results: The trial completed with 27 patients with peritoneal carcinomatosis secondary to gynecological malignancies (n=14), gastrointestinal malignancies (n=12), and peritoneal mesothelioma (n=1). The starting dose level was 35mg/m2 and escalated to 170mg/m2. The maximum tolerated dose (MTD) of IP nab-paclitaxel was established at 140 mg/m2, while to the MTD of weekly IV nab-paclitaxel was established at 100 mg/m2 in heavily pretreated patients and 150 mg/m2 for the lightly pretreated group. Drug limiting toxicities included grade 3 neutropenia resulting in treatment delay >15 days, grade 3 abdominal pain and grade 4 neutropenia > 7 days. Fourteen patients underwent voluntary neurological exam, nerve conduction studies, and quantitative sensory nerve testing at baseline and at pre-cycle three. Although there was a slight trend towards worsening neuropathy, no significant differences were found for any of the measured parameters.
Discussion: IP *nab*-paclitaxel achieved therapeutic levels in the peritoneum and systemically, without causing significant objective or subjective neuropathy. This is in contrast to other forms of taxane-based chemotherapies and IV *nab*-paclitaxel, for which neuropathy is a common treatment-limiting toxicity**.**
Conclusion: IP *nab*-paclitaxel has a favorable neuro-toxicity profile and can be safely administered in patients with prior exposure to paclitaxel.

Disclosures: N. Prakash: A. Employment/Salary (full or part-time): City of Hope Medical Group. **C. Mihaela:** A. Employment/Salary (full or part-time): City of Hope Medical Group. **P. Frankel:** A. Employment/Salary (full or part-time): City of Hope. **H. Openshaw:** None. **R. Morgan:** A. Employment/Salary (full or part-time): City of Hope Medical Group.

Poster

706. Pain Models: Human Studies

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 706.09/BB15

Topic: D.02. Somatosensation: Pain

Support: NIH (EB00856)

NIH (EB006356)

NIH (EB 018783)

W911NF-08-1-0216

W911NF-12-1-0109

W911 NF-14-1-0440

Title: Adaptive neurotechnologies for improved management of chronic pain

Authors: *S. N. JOSHI[1], A. DE PESTERS[1,2], P. BRUNNER[1,3], J. P. CLEARY[4], J. FUDIN[4,5,6], G. SCHALK[1,2,3];
[1]Natl. Ctr. For Adaptive Neurotechnologies, Albany, NY; [2]State Univ. of New York at Albany, Albany, NY; [3]Dept. of Neurol., Albany Med. Col., Albany, NY; [4]Stratton VA Med. Ctr., Albany, NY; [5]Remitigate, LLC, Delmar, NY; [6]Western New Eng. Univ. Col. of Pharm., Springfield, MA

Abstract: Chronic pain costs billions of US dollars yearly. Pharmacologic treatment options are expensive and have treatment barriers that include neurotoxicity, gastrointestinal bleed, kidney dysfunction, cardio-pulmonary toxicities, opioid addiction sequalae, and opioid related concerns that include tolerance, dependence, respiratory depression and various public health detriments. Recent recognition of the remarkable plasticity of the nervous system opens up the exciting possibility to develop systems that establish real-time adaptive interactions with the nervous system to improve functions impaired by injury or disease. In the context of chronic pain, these systems, called adaptive neurotechnologies, could induce beneficial neuroplasticity that reduces pain perception and thus creates an entirely new, non-pharmaceutical, approach to management of chronic pain.
The possibility for this approach to pain modulation is further supported by the growing recognition that pain may not be proportional to peripheral nociceptive input alone. Indeed, it is now well known that nociceptive input is modulated at multiple levels in the brain, including subcortical and cortical areas, and nociception may neither be sufficient nor necessary for the experience of chronic pain (Moseley, 2003, Jensen et al., 2008). Different functional imaging techniques provide a window into these modulatory brain processes. For example, in subjects

experiencing acute pain, specific frequencies in electroencephalographic (EEG) signals are related to the magnitude of the experience of pain. Specifically, many studies have shown that reduced power in the alpha (8-12 Hz) band is associated with increased experience of acute pain (Jensen et al. 2008) that may be due to increased transfer of nociceptive information through thalamo-cortical and cortico-cortical channels (Jacobs et al. 2015).
Our overall goal is to develop, implement and validate a novel adaptive neurotechnology to improve the management of chronic pain. In the present pilot study, we hypothesize that, for patients with chronic pain in their extremities, the power of sensorimotor alpha is negatively related to pain perception, that patients can learn to associate pain perception with an increase in alpha power, and that this change in brain function reduces perception of chronic pain in the extremities.
To date, we have studied 3 patients who underwent non-invasive monitoring of scalp EEG. Initial results show that alpha power is reduced on the hemisphere contralateral to the painful extremity, and that contralateral alpha power is decreased further during attention to or stimulation of the painful hand.

Disclosures: **S.N. Joshi:** None. **A. de Pesters:** None. **P. Brunner:** None. **J.P. Cleary:** None. **J. Fudin:** A. Employment/Salary (full or part-time): Stratton VA Medical Center; Albany NY (Full Time employee). 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; PI, CASE SERIES DESCRIBING TAPENTADOL USE IN PATIENTS ON HEMODIALYSIS. This is at VA above but not funded. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); None. D. Fees for Non-CME Services Received Directly from Commercial Interest or their Agents (e.g., speakers' bureaus); Speaking Engagements; Astra Zeneca, DepoMed, Endo, Kaléo, Millennium Health LLC, Pernix. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); CEO and Owner of Remitigate LLC. F. Consulting Fees (e.g., advisory boards); Consulting/Advisory Boards; Astra Zeneca, DepoMed, Endo, Kaléo, Kashiv Pharma, KemPharm, Scilex Pharmaceuticals. Other; Not applicable. **G. Schalk:** None.

Poster

706. Pain Models: Human Studies

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 706.10/BB16

Topic: D.02. Somatosensation: Pain

Support: Endo Pharmaceuticals

Title: Topical Lidoderm® (lidocaine 5% patch) alters the density of sodium channel subunits in epidermal keratinocytes among patients with diabetic neuropathy

Authors: *P. J. ALBRECHT[1], C. E. ARGOFF[2], J. P. WYMER[3], F. L. RICE[1];
[1]Integrated Tissue Dynamics, LLC, Rensselaer, NY; [2]Dept. of Neurol., Albany Med. Ctr., Albany, NY; [3]Dept. of Neurol., Univ. of Florida Col. of Med., Gainsville, FL

Abstract: According to the NIH, in 2012 there were close to 30 million people in the United States with diabetes, effecting men and women equally, and estimates of up to 1 in 5 of those patients (roughly 6 million individuals) experience painful diabetic neuropathy (PDN). The purpose of this research study was to document the extent of topical lidocaine, administered via Lidoderm®, to provide therapeutic benefit to patients suffering with PDN, and to determine if epidermal keratinocyte expression of voltage-gated sodium channel subunits was altered in patients compared with controls, and to document if the expression was changed following patch treatment. The final cohort of study contained 21 painful diabetic neuropathy patients, 12 non-painful diabetic neuropathy patients, and 11 healthy control subjects. Each of the subjects underwent a detailed quantitative sensory testing (QST) battery, followed by a 3mm cutaneous punch biopsy, application of the Lidoderm® 5% patch for 4-weeks, followed by a subsequent QST and punch biopsy. Clinical reporting measures were maintained to determine responders from non-responders, and immunochemical evaluations were performed on the pretreatment and post-treatment biopsies to determine innervation and voltage-gated sodium channel expression changes. Lidoderm® provided substantial analgesic relief to painful diabetic patients. Evaluations of the cutaneous innervation revealed that no changes among the epidermal, subepidermal, or upper dermal compartments were detected that corresponded with the response to, or effectiveness of, the treatment. In contrast, a significant decrease in keratinocyte expression of Nav1.6 correlated with reduced painfulness after treatment, whereas keratinocyte Nav1.7 expression remained generally unaltered. The results indicate that the use of Lidoderm® to treat painful peripheral neuropathy may involve mechanisms associated with keratinocyte expression of sodium channel subunits, and that epidermal keratinocyte expression of specific Nav subunits may serve as a predictor of patch treatment efficacy.

Disclosures: **P.J. Albrecht:** None. **C.E. Argoff:** None. **J.P. Wymer:** None. **F.L. Rice:** None.

Poster

706. Pain Models: Human Studies

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 706.11/DP04 (Dynamic Poster)

Topic: D.01. Sensory Disorders

Support: NIH grant DK105687

Title: Selective stimulation of silent, mechano - insensitive C nociceptive fiber in humans?

Authors: *M. I. NEMENOV[1,2], M. KLUKINOV[2], D. C. YEOMANS[2], M. SCHMELZ[3], M. BACKONJA[4];
[1]Lasmed LLC, Mountain View, CA; [2]Anesthesia, Stanford Univ., Stanford, CA; [3]Anesthesiol., Heidelberg Univ., Mannheim, Germany; [4]Neurol., Univ. of Wisconsin, Madison, WI

Abstract: Mechanisms of painful peripheral neuropathies (PPN) are still unclear and biomarkers are not available. Abnormal spontaneous activity of C-mechano-insensitive silent fibers (CMi) is a likely source of the pain in animals and humans with PPN. Activation of CMi fibers produces axon-reflex related flare (1). However, CMi activation thresholds are substantially higher compared to C-polymodal CMH fibers which makes these fiber inaccessible for any behavioral, psychometric (QST) or cortical evoked potential tests. These high thresholds are likely due to the deeper location of CMi fibers compared to CMH fibers. Thus, assessment of CMi fibers is impossible for clinic practice, clinical research or preclinical analgesic development. We developed a diode laser C-fiber type selective stimulation (C-DLss) technique that may selectively test CMi fibers (2). DLss radiation penetrates deep into the skin, homogeneously heating superficial and subepidermal skin layers, thereby allowing access to deeply located C fibers. A decreased density of intraepidermal fibers and 50% lower density of CMH vs. CMi fibers (3) as well as sensitization to heat of CMi fibers allows for their selective activation in PPN patients (4). Here we present a modified method that may provide selective activation of CMi vs. CMH fibers in healthy subjects. We hypothesized that controlled cooling of skin surface allows to temporarily decrease response from epidermal C fibers mimicking functional denervation, though deeper located dermal CMi fibers would be spared from cooling allowing for study by DLss. Thus, selective stimulation of CMi nociceptors could be possible. The activation of CMi fibers was confirmed by generation of axon-reflex flare. The C-DLss stimuli with duration 1 s were applied to cooled (22 C°+/-1 C°) and non-cooled (32 C° +/-1 C°) inner forearm skin of healthy subjects. Sub- and supra pain threshold intensities did not produce warm or hot sensations in cooled skin, but only a single modality step function of pain perception. We conclude that DLss stimulation combined with superficial skin cooling provides specific noxious heat stimulation of deeper skin layers that activates CMi nociceptors.
1. Schmelz et al. Which nerve fibers mediate the axon reflex flare in human skin? Neuroreport 2000.2. Moeller-Betram et al. Sensory Small Fiber Function Differentially Assessed with Diode Laser Quantitative Sensory Testing in Painful Neuropathy. Pain Medicine 2013.3. Orstavik et al. Abnormal function of C-fibers in patients with diabetic neuropathy. J Neurosci 2006.4. Nemenov et al. Heating of deeper skin layers might detect spontaneously active heat-sensitized nociceptors. SFN 2015.

Disclosures: **M.I. Nemenov:** A. Employment/Salary (full or part-time): LasMed LLC. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); LasMed LLC. **M. Klukinov:** None. **D.C. Yeomans:** None. **M. Schmelz:** None. **M. Backonja:** None.

Poster

707. Inflammatory Pain

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 707.01/BB17

Topic: D.02. Somatosensation: Pain

Support: NIH-NIDCR #DE021888 (OJI)

UMKC-GAF 2016

Title: Oxidant-mediated release of high mobility group box 1 (HMGB1) and inflammatory cytokines in synovial fibroblasts through TLR-4 activation: Effect on cell death.

Authors: *A. A. ALSOUSI, O. J. IGWE;
Pharmacol. and Toxicology Div., Univ. of Missouri -KC, Kansas City, MO

Abstract: BACKGROUND: As a chronic autoimmune systemic disease, rheumatoid arthritis (RA) is a leading cause of disability that affects about 1% of the worldwide population, especially women. RA is characterized by synovial inflammation that lead to tissue and bone destruction. The factors that initiate, propagate and maintain RA are not clear. Reactive oxygen species (ROS) can interact with and modify cellular macromolecules, which results in structural and/or functional changes in gene and protein expressions. ROS are recognized as an important initiating factor in many diseases. Trace metals as pro-oxidants are sources of reactive species that can cause cellular oxidative stress. High mobility group box 1 (HMGB1) is a nuclear protein that is released by damaged or stressed cells. HMGB1, involved in both acute and chronic inflammatory process, is a representative damage-activated molecular pattern (DAMP) molecule that can activate TLR-4. A pathophysiological aspect of RA is an increase in the number of resident synovial cells and the resistance of fibroblasts to receptor induced apoptosis. Molecular changes in synovial fibroblasts that are induced by the exogenous pro-oxidants point to profound oxidative adaptations, which suggest their involvement in RA pathogenesis.
METHODS: We used three trace metal agents as exogenous sources of pro-oxidants. These include potassium peroxychromate (PPC) (Cr^{+5}), cuprous chloride (Cu^{+}), and ferrous chloride (Fe^{2+}), which we used to examine the biochemical interactions of pro-oxidants in HIG-82 synovial fibroblasts. HMGB1 release was measured by immunofluorescence (IF), ELISA and flow cytometry (FC). Cell death was measured by FC and WB, whereas inflammatory cytokines were quantified by ELISA.
RESULTS: The pro-oxidants increased HMGB1 release from the nucleus to the cytoplasm and upregulated its protein expression. Pro-oxidants increased release of both pro-inflammatory cytokines TNF-α, IL-1β and the anti-inflammatory cytokine IL-10. Furthermore, pro-oxidants increased proliferation of synoviocytes and appear to protect cells against apoptosis through activation of autophagy pathway.

CONCLUSIONS: Oxidant-induced TLR-4 activation caused release of HMGB1 as a pro-inflammatory response and other inflammatory cytokines such as TNF-α, which mediates pro-inflammatory actions and contribute to the pathogenesis of RA. The trace metals as pro-oxidants may protect against apoptosis by inducing autophagy while increasing synovial fibroblasts proliferation, which is in agreement with the pathophysiological changes that occur in active RA.

Disclosures: **A.A. Alsousi:** None. **O.J. Igwe:** None.

Poster

707. Inflammatory Pain

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 707.02/BB18

Topic: D.02. Somatosensation: Pain

Support: Department of Biomedical Sciences, The University of Sheffield

Title: VAMPs involved in voltage-gated cation channel trafficking in DRG neurons

Authors: ***M. ALVES SIMOES**, B. DAVLETOV, M. NASSAR, E. P. SEWARD;
Univ. of Sheffield, Sheffield, United Kingdom

Abstract: Dorsal root ganglion (DRG) neurons perceive and discriminate diverse types of sensations. Nociceptors are a subgroup of DRG neurons specialized in translating noxious pain stimuli to the spinal cord and higher brain centers. Following a noxious insult nociceptors are known to have enhanced excitability and peptide secretion (England et al., 1994; Garry and Hargreaves, 1992) both of which, are likely to be a consequence of increased membrane trafficking and vesicle fusion with the plasma membrane.
Vesicle associated membrane proteins (VAMPs) are vesicular SNARE proteins (v-SNAREs) which complex together with cognate target SNARE proteins (t-SNAREs) found on 'acceptor' compartments to regulate membrane trafficking and vesicle fusion; while much is known about the v- and t-SNAREs involved in the fusion of neurotransmitter and neuropeptide containing vesicles with the plasma membrane, to date the identity of SNAREs involved in trafficking ion channels to the plasma membrane of nociceptors is unknown.
Microarray data indicates all 7 isoforms of vamp mRNA are expressed in primary cultures of DRG neurons. Using subtype selective antibodies, we have confirmed DRG neurons express the 7 isoforms at the protein level, although our immunocytochemistry data analysis suggests differences in expression levels of specific isoforms across somata sizes.
Preliminary studies using novel engineered clostridium neurotoxins (Bitox A and Tetbot A) (Ferrari et al., 2013; Mangione et al., 2016) targeting the neuronal t-SNARE SNAP-25 suggest

that inflammation induced trafficking of voltage-gated cation channels to the plasma membrane does not use the same SNARE machinery as is required for neurotransmitter release.

Disclosures: **M. Alves Simoes:** None. **B. Davletov:** None. **M. Nassar:** None. **E.P. Seward:** None.

Poster

707. Inflammatory Pain

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 707.03/CC1

Topic: D.02. Somatosensation: Pain

Support: CIHR Research Chair

Title: Temporal contribution of the CCL2/CCR2 chemokine axis to chronic inflammatory pain elucidated by dicer substrate small interfering RNA

Authors: ***M.-A. DANSEREAU**[1], A. M. JACOBI[2], S. S. ROSE[2], M. A. BEHLKE[2], J.-M. LONGPRE[1], P. SARRET[1];
[1]Univ. De Sherbrooke, Sherbrooke, QC, Canada; [2]Integrated DNA Technologies, Coralville, IA

Abstract: There is now compelling evidence suggesting that chemokines, and especially CCL2 and its cognate receptor CCR2, contribute to the development and maintenance of chronic pain. However, due to the chemokine redundancy and lack of selective antagonists, classical pharmacological approaches did not provide specific insights into the regulation of chemokines in different chronic pain paradigms. In this study, we thus investigated the temporal contribution of the CCL2/CCR2 axis to mechanical hypersensitivity in a chronic inflammatory pain model by using specific 27-mer double-stranded dicer substrate small interfering RNA (DsiRNA). DsiRNA were encapsulated into lipid nanoparticles (LNPs) with the NanoAssemblr™ system from Precision NanoSystems Inc. LNPs containing DsiRNA targeting either CCL2 or CCR2 were intrathecally (i.t.) administered to Sprague Dawley rats at different time points before or after intraplantar administration of complete Freund's adjuvant (CFA). CFA-induced mechanical hypersensitivity was measured using von Frey hairs. Firstly, LNPs containing the Dil fluorescent dye intrathecally injected between L5 and L6 vertebrae were shown to be up-taken by L4-L6 dorsal root ganglia (DRG). However, no fluorescence staining was detected in the corresponding L4-L6 spinal cord segment. Accordingly, western blot analysis demonstrated that knockdown of the target protein by LNPs containing DsiRNA only occurred in DRG tissue. Secondly, repeated i.t. injections of 5 µg of DsiRNA against CCL2 or CCR2 48 h and 24 h before CFA administration completely prevented CFA-induced mechanical hypersensitivity up to day 5 post-

CFA. Finally, when injected 24 h and 48 h after CFA, DsiRNA against CCR2 also reverted the mechanical hypersensitivity up to day 5 post-CFA, while DsiRNA against CCL2 only exerted a significant anti-allodynic action up to day 3. Interestingly, while the mechanical hypersensitivity was present on days 7-10 post-CFA, animals treated on days 1 and 2 post-CFA with DsiRNA against either CCR2 or CCL2 exhibited a significantly higher mechanical threshold on day 14 post-CFA than rats treated with non-targeting control DsiRNA. These experiments thus reveal, through the development of nanoparticle-encapsulated DsiRNA, that the CCR2/CCL2 axis expressed at the DRG level contribute in a time-dependent manner to the generation of chronic inflammation-induced pain. These results further support the use of RNAi-based gene therapy as a highly specific alternative to classical pharmacological approaches to treat central pathologies such as chronic pain.

Disclosures: M. Dansereau: None. **A.M. Jacobi:** None. **S.S. Rose:** None. **M.A. Behlke:** None. **J. Longpre:** None. **P. Sarret:** None.

Poster

707. Inflammatory Pain

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 707.04/CC2

Topic: D.02. Somatosensation: Pain

Title: Effects of the cytokine Interferon-gamma in combination with other cytokines on slowly or fast conducting nerve fibers in rat *In vivo*

Authors: *F. RICHTER, S. WANDT, F. SLOWIK, H.-G. SCHAIBLE;
Univ. Hosp. Jena, Jena, Germany

Abstract: Interferon-γ (IFN-γ) in slowly conducting nerve fibers both in normal and in inflamed knee joints acts as an antinociceptive cytokine, whereas its effect on fast conducting fibers depends on the situation in the knee joint (inflamed or non-inflamed). In this study we investigated whether the antinociceptive effect of IFN-γ is still maintained in normal knee joints when it is applied simultaneously with or consecutively after sensitization by a pronociceptive cytokine (IL-17A or TNFα). Healthy adult WISTAR rats were anesthetized with sodium thiopentone (100 mg/kg, i.p.). The knee joint was mechanically stimulated by innocuous (20 mNm) or noxious (40 mNm) rotations of the lower leg against the fastened femoral bone for 15 sec each. Action potentials were recorded from nerve fibers that were classified as C- or as Aδ-fibers by their conduction velocity (<1.4 m/s or <10 m/s, respectively). Compounds were injected into the joint cleft at a volume of 0.1 ml each. In normal knee joints a single intraarticular injection of 10 ng of IFN-γ together with 100 ng of IL-17A decreased significantly

the net response rate of C-fibers to noxious stimulation within three hours (IFN-γ alone by 171±56, IFN-γ+IL-17A by 335±66 APs/15s, mean±SEM, respectively). The combination of IFN-γ+IL-17A decreased to a minor degree the response rates in Aδ-fibers (no change after IFN-γ alone, IFN-γ+IL-17A decrease by 76±40 APs/15s). When slowly conducting C-fibers were first sensitized by an injection of 50 ng of IL-17A (increase in net response rate to noxious stimulation within 2 hours by 42±15 APs/15s), a subsequent injection of 10 ng of IFN-γ decreased the net response rate within 1 hour by 64±26 APs/15s below control values. The utilization of the same protocol to fast conducting Aδ-fibers did not induce any changes in the response rate to either innocuous or noxious stimuli. Simultaneous application of 10 ng of IFN-γ together with 5 ng of TNFα completely prevented the sensitizing effect of TNFα in slowly and in fast conducting nerve fibers throughout the 3 hours observation period (C-fibers control 169±13, after 3 hours 179±18 APs/15s, Aδ-fibers control 379±28, after 3 hours 407±18 APs/15s, respectively). The antinociceptive effect of IFN-γ in slowly conducting nerve fibers is still maintained, when it is applied together with pronociceptive cytokines. IFN-γ is also able to desensitize these nerve fibers after previous sensitization with a pronociceptive cytokine. Therefore, IFN-γ is an important player in the precise adjustment of the different cytokines released in the joint cleft.

Disclosures: **F. Richter:** None. **S. Wandt:** None. **F. Slowik:** None. **H. Schaible:** None.

Poster

707. Inflammatory Pain

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 707.05/CC3

Topic: D.02. Somatosensation: Pain

Support: CNRS

Ecole Suprieure de Physique et chimie Industrielle

Institut de France

Title: Rac 1 dependent structural plasticity of the anterior cingulate cortex regulates the emotional aspect of chronic pain

Authors: ***S. PEZET**, M. THIBAUT, M. PALUD, Z. LENKEI;
Lab. of Brain Plasticity, Paris, France

Abstract: Painful experiences are multi-layered, composed of sensory, affective, cognitive and behavioural facets. While it is well accepted that the development of chronic pain is due to

maladaptive neuronal changes, the underlying molecular mechanisms and their relationship to the different pain modalities is still not well understood. We have previously shown that a BDNF (Brain-Derived Neurotrophic Factor) - dependent plasticity takes place in the anterior cingulate cortex in a inflammatory model of chronic pain induced by intraplantar injection of Complete Freund ‘s Adjuvant (CFA). In this brain area, BDNF is a key mechanism in the development and maintenance of the affective-emotional aspect of chronic pain, while it does not modify the sensory discrimivative aspect (Thibault et al., 2014). In the current study we explore i) the presence of structural plasticity in the ACC in this animal model and ii) the putative role of the Rac-1 signaling pathway. Using Golgi- and immune-staining for several pre- or post-synaptic components, as well as behavioural tests that explore specifically the sensory discriminative (Von Frey hairs, cold plate) or the emotional aspect of pain (passive avoidance tests to either mechanical or cold stimuli), we show that the anterior cingulate cortex is indeed the site of structural plasticity, through a Rac-1 dependent mechanism and that pharmacological blockade of Rac-1 specifically prevents the development of the emotional aspect of chronic pain.

Disclosures: **S. Pezet:** None. **M. Thibaut:** None. **M. Palud:** None. **Z. Lenkei:** None.

Poster

707. Inflammatory Pain

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 707.06/CC4

Topic: D.02. Somatosensation: Pain

Support: NHRMC Grant

Title: Cystatin c attenuates cathepsin s-evoked and par2-mediated pain and inflammation

Authors: *T. LIEU, P. ZHAO, N. BUNNETT;
Monash Inst. of Pharmaceut. Sci., Parkville, Australia

Abstract: Introduction. Extracellular proteolytic activity depends on the balance of zymogen activation, protease secretion, and the availability of endogenous inhibitors. During inflammatory and neuropathic pain, lysosomal cathepsin S is released from peripheral macrophages and spinal microglial cells, and peripheral cathepsin S can cause pain *via* biased agonism of protease-activated receptor-2 (PAR_2). Endogenous cysteine protease inhibitor cystatin C is present in extracellular fluid, but its role in regulating pain is unknown. We evaluated contributions of PAR_2 and cystatin C to cathepsin S-evoked pain.
Methods. We administered cathepsin S by intraplantar or intrathecal routes, or made intraplantar injections of macrophage activator zymosan to mice. We measured paw thickness to assess

inflammatory edema and withdrawal from von Frey filaments to assess mechanical allodynia. To determine mechanisms of cathepsin S-evoked inflammation and pain, we studied PAR_2 and cystatin C knockout (KO) mice, or treated wild-type (WT) mice with cathepsin S inhibitor Z-FL-COCHO, or substance P (SP) neurokinin 1 receptor (NK_1R) antagonist SR140,333. We used activity-based probes to assess cathepsin S activity in tissue.
Results. In WT mice, intraplantar cathepsin S induced edema and mechanical allodynia of the paw, and intrathecal cathepsin S also caused allodynia. Proinflammatory and algesic actions of peripherally and centrally-mediated cathepsin S were diminished in PAR_2-KO mice. Intrathecal SR140,333 also blunted algesic effects of intrathecal cathepsin S, consistent with a neurogenic mechanism involving PAR_2-dependent release of SP from central projections of nociceptive neurons and activation of NK_1R on spinal neurons. Doses of cathepsin S that was too low to induce inflammation and allodynia in WT mice, caused a slowly developing but marked inflammation and allodynia in cystatin C KO mice. After administration of cathepsin S, enzymatic activity in tissues, determined using an activity based-probe, was markedly higher in cystatin C KO that WT mice, consistent with the exacerbated inflammation and pain in cystatin C KO mice. Cathepsin S inhibitor attenuated inflammatory and hyperalgesic actions of zymosan, implicating a key role for endogenous cathepsin S.
Conclusions. Our results show that PAR_2 mediates proinflammatory and algesic actions of cathepsin S both in the periphery and in the spinal cord, and implicate SP and NK_1R in central mechanisms of cathepsin S-evoked and PAR_2-mediated algesia. Cystatin C attenuates proinflammatory and algesic actions of cathepsin S by suppressing cathepsin S activity in extracellular fluid and limiting activation of PAR_2.

Disclosures: **T. Lieu:** None. **P. Zhao:** None. **N. Bunnett:** Other; Takeda Pharmaceuticals Inc.

Poster

707. Inflammatory Pain

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Topic: D.02. Somatosensation: Pain

Support: NIAAA Grant 021142 (SEB)

The CH Foundation Grant 241865

TTUHSC Grant 121035

Title: Antibiotic-mediated changes in formalin-induced pain responses differ between genders in DID model

Authors: H. BLANTON[1], C. BEZBORUAH[1], D. CURTIS[1], J. MARTINEZ[1], S. BERGESON[1], *J. GUINDON[2];
[2]Dept. of Pharmacol. and Neurosci., [1]Texas Tech. Univ. Hlth. Sci. Ctr., Lubbock, TX

Abstract: Inflammatory pain is a condition in which tissue becomes over sensitized and produces an exaggerated pain response to both painful stimuli, and in the case of allodynia, otherwise innoculous stimuli. Inflammatory pain can be induced through a variety of mechanisms and treatment can be difficult due to the complex signaling mechanisms that mediate the state, examples of which include prostaglandins, growth factors, and inflammatory cytokines. Inflammatory cytokines, such as TNF-alpha, are often released in response to chronic alcohol consumption, a common cause of inflammatory pain. Anti-inflammatory agents such as NSAIDs are among the most common treatments for inflammatory pain, but present problems such as liver toxicity when co-administered in the presence of chronic alcohol consumption. Novel approaches for treating inflammatory pain are now being investigated through the repurposing of readily available medications such as antidepressants, anxiolytics, and as is the case in this study, antibiotics. Aside from their main application as antibiotics, the tetracycline class of compounds has demonstrated anti-inflammatory properties. The goal of this study was to evaluate the efficacy of a synthetic tetracycline derivative as an anti-nociceptive agent in the formalin model (10ul at 2.5 % i.pl.) of pain using male and female mice in both chronic-drinking and non-drinking conditions. The results demonstrate a polarizing effect on pain perception between male and female mice across all experimental conditions. Females demonstrated the lowest pain threshold when exposed to the combined treatment of tetracycline and alcohol while males demonstrated the opposite effect. Males in the combined treatment were the least sensitive to pain of any condition. These results raise questions as to whether gender differences influence the efficacy of tetracyclines as treatments for pain and if so, what the mechanism behind these differences may be.

Disclosures: H. Blanton: None. **C. Bezboruah:** None. **D. Curtis:** None. **J. Martinez:** None. **S. Bergeson:** None. **J. Guindon:** None.

Poster

707. Inflammatory Pain

Location: Halls B-H

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Program#/Poster#: 707.08/CC6

Topic: D.02. Somatosensation: Pain

Support: KAKENHI(Multi-year Fund) Grant-in-Aid for Scientific Research (C, 16K08995)

Title: Analgesic effects of sialidase in inflammatory pain: the role of gangliosides in nociceptive signaling.

Authors: *S. WATANABE, K. TAKANO, M. SAGAWA, T. IWAI, Y. IKEDA-MATSUO, Y. NAITO, M. TANABE;
Dept. of Pharmacol. Sch. of Pharm., Kitasato Univ., Tokyo, Japan

Abstract: Gangliosides play essential roles in nervous system functions through regulating membrane organization. Gangliosides are sialic acid containing glycosphingolipids synthesized from ceramide and divided into four groups (asialo-, a-, b-, and c-series gangliosides) based on their biosynthetic pathway. Handa et al. revealed that *St8sia1* knockout mice, which lack b- and c-series gangliosides but not asialo- and a-series gangliosides, show altered nociceptive behavior (1). However, the molecular mechanism underlying pain defects remains unknown. To address this issue, we first examined whether gangliosides in skin tissues are involved in nociception. Intraplantar injection of b-series ganglioside GT1b, but not a-series ganglioside GM1, produced nociceptive behavior and enhanced 0.05% formalin-induced nociception (GT1b-induced hyperalgesia) in ICR mice. GT1b-induced hyperalgesia was blocked by glutamate receptor antagonists and elimination of glutamate in subcutaneous tissues using glutamate degradation enzymes. Furthermore, subcutaneous microdialysis analysis revealed that intraplantar GT1b injection increased glutamate concentration. These findings suggested that GT1b increased extracellular glutamate concentration in subcutaneous tissues, thereafter activating peripheral glutamate receptors, which in turn resulted in nociceptive behavior and hyperalgesia (2).
When compared with a-series gangliosides, b-series gangliosides have two sialyl residues linked to the galactose in the second position from ceramide. Therefore, sialyl residue seems to be important in nociceptive behavior after intraplantar injection of gangliosides. We examined the effects of sialidase, which degrades sialyl conjugates including gangliosides, in nociceptive behavior. One day after intraplantar injection of complete Freund's adjuvant, sialidase from *Arthrobacter Ureafaciens* was injected into the same plantar area. Indeed, sialidase treatment reduced mechanical allodynia caused by complete Freund's adjuvant induced inflammation. Consistent with analgesic effects of sialidase, intraplantar injection of GT1b into naïve mice induced mechanical allodynia. Furthermore, lipid extracts from inflammatory skin tissues but not naïve skin included complex gangliosides. These results suggested that inflammation leads to increased complex gangliosides in skin tissues, which is involved in inflammatory pain.
(1) Handa Y et al., (2005) Pain, 117, 271-279.
(2) Watanabe S et al., (2011) Pain, 152, 327-334.

Disclosures: S. Watanabe: None. **K. Takano:** None. **M. Sagawa:** None. **T. Iwai:** None. **Y. Ikeda-Matsuo:** None. **Y. Naito:** None. **M. Tanabe:** None.

Poster

707. Inflammatory Pain

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 707.09/CC7

Topic: D.02. Somatosensation: Pain

Support: AR047410

Title: The effects of hypoglutamatergic tone on physiological nociception and inflammatory pain:evaluation of glutaminase type 1 mutant Sprague-Dawley rats

Authors: *Z. ZHANG, S. DAS, M. B. ANDERSON, K. E. MILLER;
Anat. & Cell Biol., Oklahoma State Univ-CHS, Tulsa, OK

Abstract: Increased glutamatergic tone contributes to peripheral sensitization during tissue inflammation. Glutaminase type 1 (GLS1) is the synthetic enzyme that converts glutamine to glutamate in the nervous system. Several pharmacological studies have demonstrated the significant role of GLS1 in maintaining high glutamate level during acute and chronic inflammatory pain. In the current study, GLS1 heterozygous knockout ($GLS1^{+/-}$) rats were custom made out from outbred Sprague-Dawley rats, generating a rat model with hypoglutamatergic tone. Inflammatory pain was measured during an adjuvant-induced arthritis (AIA) model. A 2 × 2 design was made with genotype (WT vs $GLS1^{+/-}$) and inflammation (naïve vs AIA) as the two variables. Four experimental groups were generated (WT-naïve, WT-AIA, $GLS1^{+/-}$-naïve and $GLS1^{+/-}$-AIA). Behavioral, histopathological and molecular analyses were performed. $GLS1^{+/-}$ rats have no detectable alterations in anatomical or histological structures compared to the WT littermates. Under physiological conditions, $GLS1^{+/-}$ rats have decreased GLS1 expression in the dorsal root ganglion (DRG) neurons and peripheral tissues, but normal nociception compared to the WT littermates. When evaluating the inflammatory pain, $GLS1^{+/-}$-AIA rats developed less sensitization compared to WT-AIA rats. Both WT and GLS mutants had increased GLS1 in the associated lumbar DRG after AIA. However, the magnitude of elevation between $GLS1^{+/-}$-naïve and $GLS1^{+/-}$-AIA rats was smaller than those of WT littermates. The characterizations of $GLS1^{+/-}$ rats help us understand the role of GLS1 in maintaining glutamatergic tone. These results support that glutamatergic signaling has various contributions in physiological nociception and inflammatory pain.

Disclosures: **Z. Zhang:** None. **S. Das:** None. **M.B. Anderson:** None. **K.E. Miller:** None.

Poster

707. Inflammatory Pain

Location: Halls B-H

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Program#/Poster#: 707.10/CC8

Topic: D.02. Somatosensation: Pain

Support: CB-2012/179294

Title: Expression of anoctamin-1 and bestrophin-1 in inflammatory and neuropathic pain models in rats

Authors: *I. VELAZQUEZ LAGUNAS, J. B. PINEDA-FARIAS, V. GRANADOS-SOTO; Farmacobiología, Cinvestav, Sede Sur, Ciudad de Mexico, Mexico

Abstract: Intracellular chloride (Cl^-) concentration could increase in primary sensory neurons when exposed to inflammatory mediators or after nerve injury. Such increase modifies the Cl^- electrochemical potential and turns it more positive. In this way, calcium-activated chloride channels (CaCCs) activation produces a Cl^- efflux that under these particular conditions might facilitate the nociceptive terminals depolarization contributing to the generation of action potentials. Previous studies have suggested the participation of bestrophin-1 and anoctamin-1, members of the CaCCs, in inflammatory and neuropathic pain, but their regulation during these states are unclear. The aim of this investigation was to study the expression of anoctamin-1 and bestrophin-1 in inflammatory and neuropathic pain models. Experiments were carried out in adult female Wistar rats (body weight 140-220 g). Rats received an intraplantar injection of vehicle (saline or 10% ethanol, 10% Tween 20, and 80% saline), formalin (1%), bradykinin (10 nM), serotonin (0.1%) or capsaicin (0.1%) in to the right hind paw or spinal nerve ligation. Bestrophin-1 and anoctamin-1 mRNA and protein expression were determined in the lumbar spinal cord and dorsal root ganglia (DRG) in *naïve*, injected (1 h, 1, 3 and 6 days) and neuropathic rats (1, 7, 14 days) by PCR, Western blot and Immunofluorescence, respectively. Bestrophin-1 and anoctamin-1 mRNA and protein were expressed in the lumbar spinal cord (SC) and DRG of all groups. Intraplantar inject of formalin, bradykinin, serotonin and capsaicin increased mRNA and protein expression of bestrophin-1 and anoctamin-1 compared to the *naïve* group in the SC and DRG from 1^{st} to 6^{th} day after injection. Nerve ligation increased mRNA and protein expression of anoctamin-1, but not bestrophin-1, at the SC and DRG. Immunofluorescence showed that bestrophin-1 is expressed in non-peptidergic fibers and microglia at outer laminas of the dorsal horn SC. Moreover, the expression of bestrophin-1 and anoctamin-1 was observed in small to medium sized peptidergic and non-peptidergic DRG neurons. Results suggest that bestrophin-1 and anoctamin-1 are present in sites involved in the transmission of nociception and their expression is being modulated under inflammatory and

neuropathic pain conditions. Likewise, the data suggest that the CaCCs may represent a possible therapeutic target to develop analgesic drugs.

Disclosures: I. Velazquez Lagunas: None. **J.B. Pineda-Farias:** None. **V. Granados-Soto:** None.

Poster

707. Inflammatory Pain

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 707.11/CC9

Topic: D.02. Somatosensation: Pain

Title: Increase of t-type ca^{2+} current underlies protease-activated receptor 2-dependent sensitization of rat isolectin-b4 positive nociceptor

Authors: *S. CHUNG[1,2], Y.-H. KIM[3], J.-H. JOENG[3];
[1]Dept. of Physiology, Yonsei Univ. Col. of Med., Seoul, Korea, Republic of; [2]Brain Korea 21 Project for Med. Science, Yonsei Univ. Col. of Med., SEoul, Korea, Republic of; [3]Dept. of Physiology, Yonsei Univ. Col. of medicine, Seoul, Korea, Republic of

Abstract: The protease-activated receptor (PAR)2 family, a group of G-protein coupled receptors, is expressed on peripheral sensory neurons. Its activation evokes mechanical hyperalgesia; however, its cellular mechanism is still unknown. Recently, it has been reported that T-type Ca^{2+} channels contribute to nociception, and augmentation of the T-type calcium current (I_{Ca-T}) may evoke hyperalgesia by sensitizing peripheral nociceptors. Therefore, we hypothesized that PAR2-induced pain may be due to sensitization of primary sensory DRG neurons evoked by augmentation of I_{Ca-T}. Trypsin (30 nM) and PAR2-activating peptide H-Ser-Leu-Ile-Gly-Arg-Leu-NH_2 (SL-NH_2) (100 μM) augmented I_{Ca-T}. In current-clamp experiments, PAR2 agonists increased excitability by increasing after-depolarizing potentials (ADP)s, lowering the threshold for action potential (AP) firing. These effects were completely reversed to the control levels by application of mibefradil, (1 μM), a potent T-type channel blocker. In addition, intraplantar (i.pl.) injection of PAR2 agonists induced a prolonged mechanical hyperalgesia and elevated spinal c-Fos protein expression. These effects were prevented by co-treatment with mibefradil (5 μg/paw). These observations suggest that PAR2 activation readily augments I_{Ca-T} in peripheral nociceptive neurons, and this augmentation of I_{Ca-T} may be involved, at least in part, in PAR2-evoked mechanical hyperalgesia.
This research was supported by the Pioneer Research Center Program through the National Research Foundation of Korea funded by the Ministry of Science, ICT & Future Planning (2012-0009525) and a faculty research grant of Yonsei University College of Medicine for 2006 (6-

2011-0164).
Key words: protease-activated receptor; T-type Ca^{2+} channel; mechanical hyperalgesia; inflammation; peripheral sensitization; nociceptor

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

Poster

707. Inflammatory Pain

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Topic: D.02. Somatosensation: Pain

Support: NIH Grant AR047410

Title: The role of mir23b and let-7a in glutamatergic myenteric innervation in trinitro-benzene-sulphonic acid-induced colitis in rats.

Authors: S. DAS[1], Z. ZHANG[1], M. B. ANDERSON[1], B. GREENWOO-VAN MEERVELD[2], *K. E. MILLER[1];
[1]Dept Anat. & Cell Biol, Oklahoma State Univ. Ctr. for Hlth. Sci., Tulsa, OK; [2]Univ. of Oklahoma Hlth. Sci. Ctr., Oklahoma City, OK

Abstract: Chronic inflammatory bowel disease such as colitis is characterized by abdominal pain and pharmacological therapies are very limited. At the same time, the excessive and exacerbated immune response within the gastrointestinal tract makes the underlying mechanisms of colitis more complex to understand. Our previous finding showed elevated levels of glutaminase (GLS) in dorsal root ganglion (DRG) neurons in TNBS-induced colitis in WT rats. The incomplete understanding of mechanisms of gene expression led us to investigate the interaction between the inflammatory and neuronal responses affecting GLS in WT and GLS+/- animals. Experimentally, we induced colitis in Sprague-Dawley WT and GLS+/- rats by infusing TNBS into the colon and collected the colon and DRG (S1 and L6) at different time points. Immune hyperactivity in colon was evident in TNBS induced colitis rats with very inflamed colons. We examined miRNAs miR23b and miR-Let7a, which target GLS and nerve growth factor (NGF)-β respectively. TNBS-induced colitis decreased the expression of miR23b 5p as well as 3p in both WT and GLS+/- rats at day2. This decrease in miRNA levels corresponded with increase in GLS protein expression in TNBS-induced colitis. The expression of Let7a-5p on the other hand increased in this colitis model. We are currently investigating to find the NGF β protein expression levels to relate with Let7a results. Further, we investigated if the change in miRNA profile in colon affected the myenteric ganglia. Immunohistochemical analysis showed

that during TNBS-induced colitis there were visibly lower concentration and number of ganglia in WT animals and the ganglia were not as defined and structured as they were in control animals. GLS inhibitor, 6-diazo-5-oxo-L-norleucine (DON), pretreatment rescued the effect of colitis on these ganglia. Nevertheless, in GLS+/- animals, TNBS-induced colitis had very minimal effect on these myenteric ganglia. Their number and intensity did not change and the DON pretreatment had no effect at all on these myenteric ganglia in TNBS-induced colitis. Based on these results, it appears that GLS plays a very crucial role in TNBS-induced colitis and further investigation using different miRNA as a biomarker for colitis may be crucial for its potential role in pathogenesis, diagnosis and treatment.

Disclosures: S. Das: None. **Z. Zhang:** None. **M.B. Anderson:** None. **B. Greenwoo-Van Meerveld:** None. **K.E. Miller:** None.

Poster

707. Inflammatory Pain

Location: Halls B-H

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Topic: D.02. Somatosensation: Pain

Support: NIH Grant 2R01 018156

University of New Mexico Health Sciences Center School of Medicine RAC Grant

Anesthesiology Research Fund at the University of New Mexico Health Sciences Center

Title: Understanding the role of mannose receptor activation in improved non-viral IL-10 gene therapy for peripheral neuropathic pain

Authors: *A. G. VANDERWALL[1,2], M. S. SUN[3], S. NOOR[3], J. J. SANCHEZ[3], X. O. YANG[4], L. L. JANTZIE[5,3], E. D. MILLIGAN[3,2];
[1]Dept. of Neuroscience, Univ. of New Mexico Sc, Albuquerque, NM; [2]Dept. of Anesthesiol. and Critical Care, [3]Dept. of Neurosci., [4]Dept. of Mol. Genet. and Microbiology, [5]Dept. of Pediatrics, Univ. of New Mexico, Albuquerque, NM

Abstract: Intrathecal (i.t; peri-spinal) injection of non-viral plasmid DNA (pDNA) expressing the anti-inflammatory cytokine interleukin-10 (IL-10; pDNA-IL-10) reverses chronic pain in a variety of rat models of peripheral neuropathic pain. However, methods have required repeated i.t. injections or such large doses of pDNA &/or vehicle that clinical translation would be difficult. Our group reported that the mannose receptor (MR) agonist D-mannose (D-man)

improves therapeutic efficacy of non-viral IL-10 gene therapy in rats. MR is a scavenger receptor, but is also upregulated on anti-inflammatory glia and leukocytes (e.g. macrophages), with MR-activation possibly leading to increased anti-inflammatory signaling. The mechanisms by which MR mediates enhanced IL-10 gene therapeutic efficacy are yet unclear. To explore this further, we first tested if D-man improvement of non-viral IL-10 gene therapy requires endogenous IL-10 actions. Adult male C57BL/6 wild type (WT) and IL-10 knockout (KO) mice underwent either sciatic chronic constriction injury (CCI) or sham surgery. Baseline (BL) hindpaw response thresholds to light touch stimuli (von Frey assay for allodynia) were assessed prior to surgery and after surgery (1, 2, 3, 5, 7, and 10 days, then every 4-5 days) until allodynia resolved. Both IL-10 KO & WT mice exhibited similar levels of allodynia by day 3 post-surgery, which stably persisted until spontaneous reversal by day 45. Additionally, a single i.t. co-injection (10.5µl total) of pDNA-IL-10 (3µg) with D-man (25µg) on day 5 post-surgery produced complete and long lasting reversal from allodynia within 2 days in both IL-10 KO & WT mice. These results support that D-man is an effective adjuvant for non-viral transgene uptake and therapy in mice, and that these effects are independent of endogenous IL-10. To further test the role of MR activation in the absence of IL-10, IL-10 KO mice (CCI vs. Sham) were behaviorally verified and given i.t. co-injections (day 5 post-surgery) of either pDNA-IL-10 or a control plasmid (pDNA-GFP) with either D-man or saline. As before, CCI-treated mice given D-man/pDNA-IL-10 exhibited stable reversal from allodynia. Interestingly the mice given D-man/pDNA-GFP experienced transient pain reversal, achieving near BL levels by day 3 post-injection but returning to allodynia by day 12 post-injection. Overall these results point to an IL-10 independent mechanism of MR-mediated enhancement of initial transgene uptake, expression, and pain reversal. Ongoing studies will identify the anatomical region of transgene expression and the possible transcriptional regulation following MR-activation on glial-immune phenotypic profiles.

Disclosures: **A.G. Vanderwall:** None. **M.S. Sun:** None. **S. Noor:** None. **J.J. Sanchez:** None. **X.O. Yang:** None. **L.L. Jantzie:** None. **E.D. Milligan:** None.

Poster

707. Inflammatory Pain

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Program#/Poster#: 707.14/DD1

Topic: D.02. Somatosensation: Pain

Support: National Research Foundation (NRF) of Korea funded by the Ministry of Education, Science, and Technology grant number 2010-0013485

Title: The inhibitory effect of peripheral delta opioid receptors (dors) in knee joint on arthritis pain

Authors: *S. MOON, E. PARK, H. SUH, H. HAN;
Col. of Med. / Korea Univ., Seoul-City, Korea, Republic of

Abstract: Systemic opioid application can be used for its strong analgesic effect. However, extensive activation of opioid receptors (ORs) beyond the target can cause unwanted complications e.g. dysphoria, pruritis, respiratory depression and constipation. Therefore, the selective activation of peripheral ORs present in the afferent fibers of the targeted tissue can be considered a better strategy in opioid analgesia to avoid unwanted complications. The purpose of this study was to clarify the role of peripheral delta opioid receptors (dORs) in arthritic pain for the possible use of peripheral ORs in anti-nociception. We investigated whether the activation of peripheral dORs could reduce the nociceptive behavior and the response of sensitized primary mechanosensitive afferents (MSA) after carrageenan-induced arthritis in male Sprague-Dawley rat. Administering SNC80 (selective agonist of dOR) into the knee joint, we evaluated the nociceptive behavior by using dynamic weight bearing test which can measure the decreased weight load on affected side during freely walking, and recorded MSA activity (maximal spikes/second) in knee joint by using extracellular single nerve recording technique before and after the injection of SNC80. Four hours after the induction of arthritis, the intra-articular application of SNC80 (1μM, 100μM and 1mM) resulted in significant recovery in nociceptive behavior. Consistent with these behavioral results, we also found that the intra-articular application of SNC80 (1μM and 100μM) significantly decreased MSA activities stimulated by von Frey filaments (6 and 26g) 10 to 60 min post-drug injection. However, the application of 10nM SNC80 had no effect on MSA activities and nociceptive behavior. These results implicate that the activation of peripheral dOR in the knee joint can contribute to reducing the nociceptive behavior by decreasing the activity of MSA in inflamed knee joint.

Disclosures: **S. Moon:** None. **E. Park:** None. **H. Suh:** None. **H. Han:** None.

Poster

707. Inflammatory Pain

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Program#/Poster#: 707.15/DD2

Topic: D.02. Somatosensation: Pain

Support: Gretel And Gordon Bootes Medical Research And Education Foundation

Title: Red light (670 nm) reduces hypersensitivity and improves tactile pathway functional integrity that is preceded by decreased neuronal cell death and reactive astrocytes following a mild T10 spinal cord injury

Authors: *D. HU, J. R. POTAS;
Eccles Inst. of Neurosci., The Australian Natl. Univ., Acton, Australia

Abstract: Spinal cord injured patients often suffer from serious sensory deficits including neuropathic pain and/or loss of sensation. We investigated the effects of a 670 nm LED 30 min daily treatment on the development of neuropathic pain and tactile sensory pathway deficits in a hemi-contusion model in male Wistar rats. A spinal cord injury (hemi-contusion: 10 g, 25 mm height, diameter 1 mm) was induced on the right dorsal surface of the spinal cord at the T10 vertebral body and with the dura removed. We first assessed the development of hypersensitivity using a novel behavioural assessment assay applied to 6 regions over the rat dorsum at dermatomes above, at, and below the level of the injury and on ipsilateral and contralateral sides. Weights were applied to four behavioural categories to give a regional sensitivity score (RSS) to 10 consecutive innocuous mechanical stimulations (bending force: 2.86 ± 0.09 g) in each region. An accumulative sensitivity score (ASS) was then calculated by the sum of all 6 RSSs. Uninjured intact animals were assessed to define a hypersensitivity threshold (2 standard deviation above the mean). More than 50% of sham treated animals had ASS exceeding the hypersensitivity threshold between 1-5 days post injury (dpi) while this was reduced to 30% in 670 nm treated group. Treatment also decreased ASS in normosensitive animals compared to sham treated animals at 1-3 dpi. To study tactile pathway changes following spinal cord injury and treatment, local field potentials were measured from the brainstem surface by electrically stimulating sural nerves to ascertain the integrity of the dorsal column pathways. Sham treated animals displayed increased latency and decreased magnitude and duration of responses arising from the ipsilateral dorsal column pathway compared to the contralateral side. 670 nm treatment reversed these functional deficits, particularly at 7 dpi. Expression of astrocyte activation (GFAP) occurred from 1 dpi and was reduced by 670 nm from 3-7 dpi ipsilateral to the injury. The treatment however, did not affect IL-1β expression by astrocytes or microglia (IBA1) between 1-5 dpi. The density of iNOS containing microglia was reduced by 670 nm treatment at 7 dpi. While both groups showed reduced areas of myelination (MBP) and neurofilament density (NF200) ipsilateral to the injury, 670 nm treatment significantly reduced neuronal cell death (NeuN/TUNEL) at 1 dpi. We conclude that 670 nm reduces the early incidence of hypersensitivity, followed by improved tactile pathway integrity. Functional improvements are preceded by reduced astrocyte activation and neuronal cell death, and coincide with reduced iNOS secretion by microglia at 7 dpi.

Disclosures: D. Hu: None. **J.R. Potas:** None.

Poster

707. Inflammatory Pain

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Program#/Poster#: 707.16/DD3

Topic: D.02. Somatosensation: Pain

Title: Stimulus-evoked and spontaneous pain in Complete Freund's adjuvant -induced arthritis in the rat

Authors: *S. WAGNER, J. BINDLER, L. BOURGOIN, E. ANDRIAMBELOSON; NEUROFIT, ILLKIRCH, France

Abstract: Intra-articular injection of complete Freund's adjuvant (CFA) in rodents is a well characterized model of monoarthritis and is routinely used for the screening of compounds targeting inflammatory pain. The injection of CFA usually results in an increased sensitivity to noxious stimuli such as heat or mechanical pressure. However, there is a paucity of data concerning the time course of spontaneous pain following CFA injection. In the present study, CFA was injected into the tibio-tarsal joint of the rat hindpaw. The time course of nociceptive responses to mechanical pressure applied to the hindpaw palm (Randall & Sellito paw pressure test) was assessed on days 0, 2, 4, 7, 10, 14, 21 and 28 post-CFA injection. In addition to measuring threshold to nociceptive challenge, spontaneous pain was assessed in parallel by changes in hindpaw weight bearing using pedobarography. Significant swelling indicative of inflammation was observed in the CFA-injected (ipsilateral) paw until day 28 post-CFA although the intensity of swelling was slightly reduced from day 10 onwards. When compared to the non-injected (contralateral) paw, a significant reduction (up to 80%) of mechanical nociceptive threshold was observed in the CFA-injected paw. However, the mechanical threshold of non-injected (sham control) animals also progressively decreased with successive paw pressure tests. By day 7 (after 4 measurements), the nociceptive threshold in sham animals was equal to the level in ipsilateral of CFA injected rats. Pedobarographic measurements indicated that there was a transfer of weight bearing from the CFA-injected paw to the non-injected paw during the 3 weeks following the injection of CFA. This postural imbalance in CFA rats was maximal 4 days after CFA injection and showed up to 75% weight transfer to the non-injected paw. Whilst the imbalance remained statistically significant until day 21, recovery was evident from day 7 and complete by day 28. In contrast to mechanically evoked pain using the paw pressure test, weight bearing was not altered in sham animals even after repeated measurements. Furthermore, the data indicates that acute treatment with morphine suppressed the postural imbalanced in the hindpaws of CFA rats. From these results, pedobarography appears as unbiased measure of pain in CFA rats as compared to the mechanical pressure test. Indeed, the perception of nociceptive stimulus is influenced by the learning of the test and also by the pain status of the animal. Thus,

pedobarography could be relevant for the profiling of analgesic drugs in this animal model of arthritis.

Disclosures: **S. Wagner:** None. **J. Bindler:** None. **L. Bourgoin:** None. **E. Andriambeloson:** None.

Poster

707. Inflammatory Pain

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Title: Increased caspase1 activity in the DRG during inflammatory hyperalgesia

Authors: Y. MATSUOKA[1], K. OH-HASHI[2], *F. AMAYA[1];
[1]Kyoto Prefectural Univ. of Med., Kyoto, Japan; [2]Gifu Univ., Gifu, Japan

Abstract: Background
Caspase1, also known as interleukin1 beta converting enzyme (ICE), is the cysteine protease that mediates maturation and secretion of IL1 beta. Caspase1 is involved in the development of neuroinflammation in the central nervous system in the neurodegenerative disease. In the present study, we investigated the expression of Caspase1 and it adaptar protein ASC in the peripheral nervous system and Caspase1 activity during the inflammatory hyperalgesia.
Method
Male C57BL/6 mice (20-25g) were used for this experiments. All experimental procedures were approved by the animal ethics committee of the Kyoto Prefectural University of Medicine. Inflammatory hyperalgesia was produced by the intraplantar injection of complete Freund's adjuvant (CFA, 20μL) into the left hindpaw. Behavioral analysis including von Frey mechanical stimulation and hot plate testing were performed. For the immunohistochemistry analysis, mice treated with CFA or naïve controls were transcardially perfused with 0.9% NaCl followed by 4% paraformaldehyde in 0.1M PB under deep anesthesia. L4 and L5 DRGs were isolated and processed for immunohistochemistry. Visualization of Caspase1 and ASC was performed using fluorescent immunohistochemistry with antiCaspase1 and anti ASC antibody. For caspase1 activity measurement, mice L4 and L5 DRG were isolated Caspase1 activity was measured with the Fluorometric assay kit.

Result
In the DRG, caspase1 immunoreactivity was detected in neurons. Caspase1 expression in the DRG neurons significantly increased in CFA treated mice. Signal intensity analysis revealed that the percentage of neuron with intense signal for caspase1 significantly increased 2days after the CFA injection. The distribution of ASC was similar to Caspase1 in the DRG. Caspase1 activity of the DRG significantly increased in the CFA treated mice compared to the control.
Conclusion
Caspase1 activity in the DRG increased by the tissue inflammation. This is associated with increased expression of caspase1 in the primary afferent neurons. Caspase1 induced neuronal inflammation might be involved in the development of inflammatory hyperalgesia.

Disclosures: **Y. Matsuoka:** None. **K. Oh-Hashi:** None. **F. Amaya:** None.

Poster

707. Inflammatory Pain

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Topic: D.02. Somatosensation: Pain

Title: Receptor specific activation of sensory neurons by cytokines

Authors: ***M. GUNASEKARAN**, B. STEINBERG, T. TSAAVA, S. CHAVAN, K. TRACEY; Lab. of biomedical science, Feinstein Inst. for Med. Res., Manhasset, NY

Abstract: Memory in immune cells and neurons develops in response to environmental changes. The central nervous system modulates immunity through reflexes to monitor and defend the organism. Threat from inflammation and infection are sensed by sensory neurons activated by cytokines and other messenger molecules. Here we have shown cytokine-specific receptor expression and cytokine-mediated activation of sensory neurons from nodose ganglia (NG). Pro-inflammatory cytokines, tumor necrosis factor (TNF) and interleukin 1 beta (IL-1b) induced activation of sensory neurons revealed by measuring intracellular calcium levels with fluorescence microscopy using fluo-4 AM. Exposure of neurons from wild type mice to TNF or IL-1b significantly increased intracellular calcium levels as compared to vehicle. TNF and IL-1b at concentrations up to 100 ng/ml activated 11 % and 6 % of NG sensory neurons. Neurons from TNFR1/TNFR2 and IL1 receptor knockout mice failed to develop increased calcium levels, indicating that TNF and IL-1b induced activation is receptor specific. Together, these observations reveal a cytokine-specific and receptor-mediated activation of sensory neurons. This is a mechanism by which peripheral neural circuits sense immunological threats in a mediator specific manner.

Disclosures: **M. Gunasekaran:** A. Employment/Salary (full or part-time): Northwell health, Feinstein institute for medical research. **B. Steinberg:** None. **T. Tsaava:** None. **S. Chavan:** None. **K. Tracey:** None.

Poster

707. Inflammatory Pain

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Topic: D.02. Somatosensation: Pain

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Title: Fab fragment of Nav1.7 monoclonal antibody inhibits sodium currents in mouse and human DRG neurons and suppresses inflammatory and neuropathic pain

Authors: *R.-R. JI[1], W. CHANG[1], J. YOO[2], Y. KIM[1], G. CHEN[1], T. BERTA[1], Y. HUH[1], S.-Y. LEE[2];
[1]Pain Res. Division, Anesthesiol., [2]Biochem., Duke Univ. Med. Ctr., Durham, NC

Abstract: The voltage-gated sodium channel subtype Nav1.7 plays an important role in pain sensation in rodents and humans. Our previous study (Lee et al., Cell, 2014) showed that a monoclonal antibody that targets a Nav1.7 channel voltage sensor (SVmab1) not only suppressed sodium currents in mouse DRG neurons but also inhibited inflammatory and neuropathic pain in mouse models, compared with the control antibody (CTmab). The Fab fragment of an antibody is smaller than the whole antibody and may have better tissue penetration in vivo. In this study, we generated a Fab fragment (SVfab) derived from hybridoma-produced SVmab1 and a Fab fragment (rSVfab) derived from recombinant SVmab1. Exposure of cultured mouse and human DRG neurons to SVfab (300 and 900 nM) reduced the amplitude of transient sodium currents in a dose and time-dependent manner, whereas CTmab had no effects. The inhibition reached a plateau at 10-20 min after the SVfab incubation in DRG neurons. Notably, the inhibitory effect of SVfab is enhanced in a chronic pain condition, in which mouse and human DRG neurons were treated with the chemotherapy agent paclitaxel (1 μM). Paclitaxel also increased Nav1.7 expression and neuronal excitability in mouse and human DRG neurons. RT-PCR analysis showed that the Nav1.7 expression ratio (vs. the expression of total sodium channels) is much larger in human DRG neurons than that in mouse DRG neurons. Intrathecal injection of SVfab

reduced the formalin-induced inflammatory pain and paclitaxel-induced neuropathic pain in mice. Finally, the recombinant SVfab (rSVfab) showed similar effects in reducing sodium currents in mouse and human DRG neurons and inflammatory pain in mice. Our data suggest that the Fab antibody fragments targeting Nav1.7 also have in vitro and in vivo activities inhibiting sodium currents and inflammatory pain in mice. Since human DRG neurons express higher levels of Nav1.7 and also respond to the Fab antibody fragments, these Fab antibody fragments have the potential to be developed to treat clinical pain.

Disclosures: **R. Ji:** None. **W. Chang:** None. **J. Yoo:** None. **Y. Kim:** None. **G. Chen:** None. **T. Berta:** None. **Y. Huh:** None. **S. Lee:** None.

Poster

707. Inflammatory Pain

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Topic: D.02. Somatosensation: Pain

Support: AcRF Tier 1

Title: Antagonising LPA_1 receptor attenuates orofacial inflammatory pain in mice

Authors: ***M. SRIKANTH**, W. CHEW, S. LIM, D. R. HERR;
Dept of Pharmacol., Natl. Univ. of Singapore, Singapore, Singapore

Abstract: Lysophosphatidic acid receptor 1 (LPA_1) is one of six G-Protein Coupled Receptors (GPCRs) activated by the bioactive lipid, lysophosphatidic acid (LPA). It is highly expressed in the central and peripheral nervous systems and its signaling is essential for normal neurocortical development, among other physiological roles.
Recent studies have characterized the role of LPA_1 signaling in the pathophysiology of neuropathic pain. It has also been shown that inhibiting phospholipase A2, an enzyme that produces LPA and arachidonic acid, reduces mechanical allodynia in mice with inflammatory orofacial pain. This effect is not mediated by arachidonic acid, leading us to hypothesize that LPA via the LPA_1 receptor could be involved in mediating inflammatory orofacial pain. In our study, we used genetic and pharmacological approaches to block LPA_1 signaling and evaluate mechanical allodynia in a mouse model of carrageenan injection induced orofacial inflammatory pain. We found that LPA_1 -/- mice showed a significant reduction in pain response compared to wild type littermate controls. A similar significant reduction in pain response was observed in mice that were treated with a pharmacological LPA_1 antagonist. Interestingly, this effect was seen only when the antagonist was administered directly into the central nervous system.

Peripheral administration did not elicit any differences in pain behaviour. Taken together, our findings indicate that LPA_1 signaling in the central nervous system plays a key role in mediating orofacial inflammatory pain. Identifying LPA_1 as a potential therapeutic target for managing chronic pain could aid in the development of novel therapeutics with better efficacy and lesser adverse side-effects.

Disclosures: **M. Srikanth:** None. **W. Chew:** None. **S. Lim:** None. **D.R. Herr:** None.

Poster

707. Inflammatory Pain

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Topic: D.02. Somatosensation: Pain

Support: The Clinical & Translational Research Institute Seed Fund

Title: A novel role of xist in mediating sex-specific difference in pain and inflammation

Authors: *B. B. SHENODA[1], G. M. ALEXANDER[2], E. ARADILLAS LOPEZ[2], S. K. AJIT[1]; [1]Pharmacol. and Physiol., [2]Neurol., Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: Epidemiological studies demonstrate that biological sex represents an important risk factor in the development of chronic pain and autoimmune disorders in women. However, the prevalence and severity of acute inflammatory diseases are more common in men. X-inactive specific transcript (XIST) is a long noncoding RNA that is crucial for equalizing expression of X-linked genes between females and males by randomly inactivating one of the two X-chromosomes in female cells. Our previous studies showed that specific microRNAs down regulated in complex regional pain syndrome (CRPS), a female-prevalent disorder characterized by inflammation and chronic pain, can target XIST. Using molecular, biochemical and imaging approaches, we investigated the role of XIST in regulating inflammation and pain in mouse macrophages, in blood samples from CRPS patients and a rodent model of inflammatory pain. Our findings indicate a novel role for XIST in female cells beyond X-chromosome inactivation. These studies provide insights on how sex-specific mechanisms may contribute to inflammation, pain and analgesia, and would be beneficial in developing sex-specific treatment strategies.

Disclosures: **B.B. Shenoda:** None. **G.M. Alexander:** None. **E. Aradillas Lopez:** None. **S.K. Ajit:** None.

Poster

707. Inflammatory Pain

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Topic: D.02. Somatosensation: Pain

Support: US Fulbright Foundation

Title: Effects of alpha-7 nicotinic acetylcholine receptor positive allosteric modulator on proinflammatory cytokine expression and norepinephrine release following lipopolysaccharide-induced neuroinflammatory pain in mice

Authors: *M. ABBAS, S. RAHMAN;
Pharmaceut. Sci., South Dakota State Univ., Brookings, SD

Abstract: We have shown that alpha-7 nicotinic acetylcholine receptor positive allosteric modulator (PAM) controls neuroinflammatory pain (NP) phenotypes involving microglial activation in the hippocampus in lipopolysaccharide (LPS)-induced NP model in mice. Whether tumor necrosis factor (TNF)-alpha, a proinflammatory cytokine, and norepinephrine (NE) release is associated with NP phenotypes remains unknown. Here, we examined the effects of 3a,4,5,9b-Tetrahydro-4-(1-naphthalenyl)-3*H*-cyclopentan[*c*]quinoline-8-sulfonamide (TQS), an alpha-7 nAChR PAM, on microglial TNF-alpha expression and NE level following LPS-induced NP model in mice. Pretreatment of TQS (4 mg/kg) significantly reduced LPS-induced tactile allodynia and thermal hyperalgesia. Furthermore, pretreatment of TQS (4 mg/kg) reduced LPS-induced increased TNF-alpha mRNA and TNF-alpha expression in the hippocampus. Pretreatment of idazoxan, an alpha-2 adrenergic receptor antagonist, significantly reduced antiallodynic and antihyperalgesic effects of TQS. Moreover, TQS increased LPS-induced decreased NE level in the hippocampus. Taken together, these results suggest that TQS decreases LPS-induced NP by reducing TNF-alpha expression and increasing NE level in the hippocampus involving microglial alpha-7 nAChR positive allosteric modulation. Therefore, alpha-7 nAChR PAM such as TQS could be a potential drug candidate for the treatment of NP.

Disclosures: **M. Abbas:** None. **S. Rahman:** None.

Poster

707. Inflammatory Pain

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Support: CIC-UMSNH. 26.10

CIC-UMSNH. 30.2

Conacyt 182208

Title: Acute toluene exposure enhances hyperalgesia and allodynia in long lasting pain

Authors: *L. F. ORTEGA-VARELA[1], C. CERVANTES-DURÁN[2], M. A. TORRES-SANTANA[3], M. Y. GAUTHEREAU-TORRES[2];
[1]UMSNH, Lic. En Salud Publica, Morelia, Mexico; [2]Facultad de Ciencias Médicas y Biológicas "Dr. Ignacio Chávez", UMSNH, Morelia, Mexico; [3]Facultad de Químico Farmacobiología, Univ. Michoacana de San Nicolás de Hidalgo, Morelia, Mexico

Abstract: Toluene is a volatile solvent that can be found in products like thinner, paints and adhesives. Toluene misuse is an important public health problem in Mexico, mainly among street children and teenagers. Pharmacological studies indicate that this solvent produces pronociceptive effects in mice and rats, but the mechanisms involved are still unclear. On the other hand, chronic pain affects 25% to 29% of Mexican population. The purpose of this study was to evaluate the effect of acute toluene exposure on development of long-lasting secondary mechanical allodynia and hyperalgesia induced by formalin in rats. Formalin produces acute nociceptive behaviors (flinching and licking/lifting followed by long-lasting pain). Female Wistar rats (200-300 g) were placed in a static exposure chamber and exposed to toluene (6000 ppm) or air (control group) during 30 minutes. After acute exposure, rats were injected with 50 µl of 1% formalin in the dorsal surface of the right hind paw and 6 days later allodynia and hyperalgesia were evaluated in both paws. In control rats, formalin (1%), produced secondary mechanical allodynia and hyperalgesia in both paws. This was observed as a bilateral increase in paw withdrawal responses to the application of von Frey filaments (10 and 250 mN) that was significant one day after formalin injection and lasted for at least 12 days. In toluene-exposed rats, there was an increase of these nociceptive behaviors compared with control group ($P < 0.05$). These results suggest that toluene enhances secondary mechanical allodynia and hyperalgesia induced by formalin in rats, but further experiments are needed in order to elucidate the mechanisms involved in these effects.

Disclosures: **L.F. Ortega-Varela:** None. **C. Cervantes-Durán:** None. **M.A. Torres-Santana:** None. **M.Y. Gauthereau-Torres:** None.

Poster

707. Inflammatory Pain

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Topic: D.02. Somatosensation: Pain

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NIH Grant DE017813

Title: Epac-PKCalpha signaling in purinergic P2X3R-mediated hyperalgesia after inflammation

Authors: ***Y. GU**, G. LI, Y. CHEN, L.-Y. M. HUANG;
Univ. Texas Med. Br., Galveston, TX

Abstract: Sensitization of purinergic P2X3 receptors (P2X3Rs) is a major mechanism contributing to injury-induced exaggerated pain responses. In a previous study, we have showed that cAMP-dependent guanine nucleotide exchange factor 1 (Epac1) in rat sensory dorsal root ganglia (DRGs) is upregulated after inflammatory injury and plays a critical role in P2X3R sensitization by activating protein kinase C epsilon (PKCε) inside cells. In contract of PKCε, which has been established as the major PKC isoform mediating injury-induced hyperalgesic responses, the role of protein kinase C alpha (PKCα) in receptor sensitization was seldom considered. We therefore studied the participation of PKCα in Epac signaling in P2X3R-mediated hyperalgesia. Following complete Freund's adjuvant (CFA)-induced inflammation, we found that the expression of both Epac1 and Epac2 and the level of cAMP in DRGs are greatly enhanced. The expression of phosphorylated PKCα (pPKCα) is also upregulated. CFA-induced P2X3R-mediated hyperalgesia is not only blocked by Epac antagonists, but also by the classical PKC isoform inhibitors, Go6976, and PKCα-siRNA. These CFA effects can be mimicked by an application of the Epac agonist, CPT, in control rats, further confirming the involvement of Epacs. Since CPT can increase the expression of pPKCα and the application of Go6976 prior to CPT still reduces CPT-induced hyperalgesia, PKCα is downstream of Epacs to mediate the enhancement of P2X3R responses in DRGs. The pattern of translocation of PKCα inside DRG neurons in response to CPT or CFA stimulation is distinct from that of PKCε. Thus, in contrast to prevalent view, PKCα also plays an essential role in producing complex inflammation-induced receptor-mediated hyperalgesia.

Disclosures: **Y. Gu:** None. **G. Li:** None. **Y. Chen:** None. **L.M. Huang:** None.

Poster

707. Inflammatory Pain

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Topic: D.02. Somatosensation: Pain

Support: NIH IRP, Clinical Center

Title: The chronic pain transcriptome: RNA-Seq profiling of dorsal spinal cord during persistent inflammatory hyperalgesia.

Authors: ***M. R. SAPIO**, M. J. IADAROLA, D. M. LAPAGLIA, A. J. MANNES;
NIH, Bethesda, MD

Abstract: Persistent pain states engage new gene expression programs in nociceptive circuits in the superficial laminae of the dorsal horn. These programs occur in concert with synaptic rearrangement and serve to protect injured body parts by changing the input-output functions of the spinal cord to respond appropriately to stimulation. In pain disorders these normally adaptive alterations create inappropriate and deleterious responses. Identifying the molecular processes that are engaged during this transition provides insight into intervention. RNA-Seq provides accurate quantitation and comprehensive assessment of transcript levels of all genes expressed in a cell or tissue. This provides a broad view of all biological processes that engage the transcriptional machinery, including the engagement of targetable receptors, and the regulation of signaling molecules altered by the remodeling of the spinal cord. We examined differentially expressed genes at 2, 24 and 48 hours after inflammation to characterize the transition from normal sensation to profound hyperalgesia. Elevated expression of multiple transcription factors occurred at 2hrs with return to control levels by 48 hrs. These included known transcription factors c-Fos, the Fos paralog Fra2, and JunB, and 3 paralogs of NGFI-B (nuclear receptor family). Several neuropeptide transcripts displayed long-term increases including prodynorphin, proenkephalin and protachykinin, as expected. In addition, many of the most highly expressed and highly differential genes are immune-like and are part of the microglial complement system, which engages in degrading synaptic connections to change the circuitry of the spinal cord. This induction occurs in the absence of other inflammation markers, and is long-lived. We further examined to what extent these genes are expressed in different cell types and tissues, incorporating additional information from large public datasets. We conclude that neuronal activity is accompanied by complement-dependent synaptic rearrangement by microglia, and that a relatively discrete set of receptors and their ligands are regulated by peripheral inflammation.

The data demonstrate that a cadre of state-dependent drivers and downstream effector molecules are induced to control nociceptive primary and secondary sensitization processes that occur in persistent pain states, and describe in a comprehensive fashion the cascade of signals involved in the transition to persistent pain.

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Poster

707. Inflammatory Pain

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Support: NIAAA grant 021142 (SEB)

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Title: Gender influences efficacy of tetracycline derivative at relieving formalin and alcohol-induced mechanical and cold allodynia

Authors: *H. BLANTON[1], D. HUNTER[1], C. SHERFEY[2], C. BEZBORUAH[1], J. MARINEZ[1], S. BERGESON[1], J. GUINDON[1];
[1]Texas Tech. Univ. Hlth. Sci. Ctr., Lubbock, TX; [2]Texas Tech. Univ., Lubbock, TX

Abstract: Tetracycline and its synthetic and semi-synthetic derivatives were developed as broad-spectrum antibiotics that were later experimentally demonstrated to possess anti-inflammatory activity. This anti-inflammatory action suggests potential application of tetracycline derivatives as novel alternatives for the treatment of inflammatory pain. Our study investigates the effect of a synthetic tetracycline compound on formalin-induced and chronic alcohol-induced mechanical and cold allodynia in male and female wild-type C57BL/6 mice. First, we evaluated the effect of the tetracycline compound on mechanical and cold allodynia in formalin-naïve mice with results indicating no difference from control. We then repeated the mechanical and cold allodynia tests at 60 minutes following the formalin test in control and tetracycline-treated mice. Our results demonstrated an antinociceptive effect in the mechanical and cold allodynia test values when pre-treated with the tetracycline compound, with values returning to baseline levels 2 hours after formalin injection. Finally, we evaluated the effect of chronic alcohol consumption using the DID model on mechanical and cold allodynia in control and tetracycline pre-treated mice. Our

results demonstrated gender related differences in both alcohol's effect on mechanical and cold thresholds as well as the efficacy of the tetracycline compound at mediating the alcohol-induced mechanical and cold allodynia. Further studies are needed to investigate what mechanisms, whether gender or otherwise, underlie these differences.

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Poster

707. Inflammatory Pain

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Topic: D.02. Somatosensation: Pain

Title: The effect of cutaneous inflammation on subsets of satellite glial cells in mouse dorsal root ganglion

Authors: *B. KNIGHT[1], R. M. RITZEL[2], E. R. JELLISON[2], E. E. YOUNG[3], K. M. BAUMBAUER[3];
[1]Neurosci., [2]UCONN, Farmington, CT; [3]UCONN, Storrs, CT

Abstract: Peripheral inflammation and tissue injury causes alterations in nociceptor activity and contributes to the development of normal and pathological pain states. Primary afferent nociceptors have been extensively characterized, and like central neurons, neurons that exist within the DRG do not appear to act in isolation in the peripheral pain circuitry. A resident population of peripheral glial cells, satellite glial cells (SGCs), appears to influence nociceptive activity, but the precise mechanisms by which SGCs alter neuronal function is not well understood because of difficulty in isolating adult cells without their neuronal counterparts. Ultimately, in order to understand the role of SGCs in nociceptive processing we must develop a basal cellular profile by which SGCs can be identified. To accomplish this, we used fluorescence activated cell sorting (FACS) to identify neuronal and non-neuronal cell populations in mouse DRG using CD200 and CD45, respectively. Using this broad gating strategy, we identified putative neurons (CD200+ high/CD45-), a non-neuronal cell type (CD200+ intermediate/CD45-) and two immune-related cells. We then FACS sorted SGCs based on GFAP, S100, and glutamine synthetase (GS) immunofluorescence, and found that the majority of sorted cells were GFAP+ (56.3%), while S100+ (31.3%) and GS+ (12.5%) cells constituted a lower percentages, respectively. GFAP+/S100+ and GFAP+/S100+ each represented 50% of the overlapping cell populations. Next, we examined the impact of cutaneous inflammation on SGC protein expression by injecting mice with 20 uL of CFA into the dorsal surface of one hindpaw. L2 and

L3 DRG were collected 24 hr later and cells were FACS sorted based on GFAP, S100, and GS immunofluorescence. We found that inflammation increased the the percentage of S100+ cells by 23.2%, while the percentage of GFAP+ and GS+ cells slightly decreased by 16.7% and 6.5%, respectively. When GFAP+/S100+ and GFAP+/GS+ cell populations were examined we found that inflammation caused a 50% decrease in both the GFAP+/S100+ and GFAP+/GS+ cell populations. Our data suggest the presence of a heterogeneous population of SGCs, and given the divergent regulation of SGC markers following inflammation our results further suggest there may be functional differences between the various populations of cells. Ongoing experiments are developing gene expression profiles for naïve and inflamed SGCs and are aimed further characterizing the role of SGCs in pain processing.

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Poster

707. Inflammatory Pain

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Title: Regulation of peripheral opioid receptor-mediated signaling and antinociception by 12- and 15-hydroxyeicosatetraenoic acid

Authors: *K. A. BERG[1], L. C. SULLIVAN[1], T. A. CHAVERA[1], M. PANDO[1], X. GAO[2], W. P. CLARKE[1];
[1]Pharmacol., Univ. of Texas Hlth. Sci. Ctr. at San Antonio, San Antonio, TX; [2]Metabolomics Core, Univ. of Texas Hlth. Sci. Ctr., San Antonio, TX

Abstract: Delta opioid receptors (DOR) expressed by peripheral pain-sensing neurons (nociceptors) are regulated by both cyclooxygenase (COX)- and lipoxygenase (LOX)-dependent arachidonic acid (AA) metabolites (Sullivan, Berg and Clarke, JPET 353:44-51, 2015). Whereas COX metabolites induce functional competence (i.e., responsiveness to agonist) for antinociception, LOX metabolites induce a non-responsive state of the receptor that is refractory to re-induction of functional competence. In this study, using high performance liquid

chromatography tandem mass spectrometry analyses, we have found that the 12/15-lipoxygenase AA metabolites, 12-HETE and 15-HETE, are elevated following treatment of adult rat primary sensory neuron cultures with AA. Exogenously applied 12-HETE and 15-HETE blocked DOR-mediated inhibition of PGE_2-stimulated adenylyl cyclase (AC) activity in primary sensory neuron cultures as well as DOR-mediated reduction of PGE_2-evoked thermal allodynia in the rat hindpaw. In addition, treatment of primary cultures of peripheral sensory neurons with 12- and 15-HETE blocked kappa opioid receptor-mediated inhibition of PGE_2-stimulated AC but had no effect on the responsiveness of $5\text{-}HT_{1B/1D}$ receptors for inhibition of PGE_2-stimulated AC. Further, pretreatment with 12- and 15-HETE had no effect on either DOR- or KOR-mediated extracellular signal-regulated kinase (ERK1/2) activation. Taken together, these data suggest that 12- and 15-HETE selectively induce a non-responsive state of opioid receptors for antinociceptive signaling. These results exemplify the differential regulatory control of opioid receptors expressed by peripheral nociceptors and suggest that the duration of opioid receptor responsiveness under inflammatory conditions may be increased by inhibition of 12/15-lipoxygenase.

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Poster

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Title: 12- and 15-hydroxyeicosatetraenoic acid promote a non-responsive signaling state of delta opioid and kappa opioid receptors, but not for delta-kappa heteromers, in peripheral nociceptors

Authors: ***M. M. PANDO**, T. A. CHAVERA, W. P. CLARKE, K. A. BERG;
Univ. of Texas Hlth. Sci. Ctr. San Anto, San Antonio, TX

Abstract: Opioid receptor systems expressed by peripheral pain-sensing neurons (nociceptors) are under dual regulatory control by cyclooxygenase (COX) and lipoxygenase (LOX) dependent arachidonic acid (AA) metabolites. Neither DOR nor KOR are functionally active for

antinociception under basal conditions, but become responsive following exposure to inflammatory mediators (e.g., carrageenan, bradykinin (BK) or AA) that produce COX-dependent AA metabolites. However, opioid receptors revert to a non-signaling state due to production of LOX-dependent AA metabolites, 12- and 15-HETE (see Berg et al., this meeting). Here we sought to examine the effects of LOX-dependent regulation of DOR-KOR heteromer signaling and antinociception in primary cultures of adult rat peripheral sensory neurons (ex vivo model) and in the carrageenan model of inflammation (in vivo). Ex vivo, we compared the effects of 12- and 15-HETE on inhibition of PGE_2-stimulated cAMP accumulation by the DOR agonist, DPDPE, the KOR agonist, U50488 and the DOR-KOR heteromer agonist, 6'-GNTI. Addition of 12- and 15-HETE blocked DPDPE- and U50488-mediated inhibition of PGE_2-stimulated cAMP accumulation. By contrast, 12- and 15-HETE had no effect on 6'-GNTI-mediated inhibition of PGE_2-stimulated cAMP accumulation. We next compared the abilities of DPDPE, U50488 and 6'-GTNI to inhibit carrageenan induced thermal allodynia in the rat hind paw. When administered 15 min after intraplantar (i.pl) injection of carrageenan (500 ug), all agonists completely reduced carrageenan-induced thermal allodynia. When injected (i.pl.) 3h or 24h after carrageenan administration, neither DPDPE nor U50488 produced antinociceptive responses. However, responsiveness was restored following i.pl. injection of the 12/15-LOX inhibitors, baicalein and luteolin. Interestingly, the heteromer agonist, 6'-GNTI, completely inhibited the nociceptive response when administered (i.pl.) 3h or 24h (longest period tested) after carrageenan. Taken together, in striking contrast to DOR and KOR, DOR-KOR heteromers appear to remain functionally competent for a prolonged period of time under inflammatory conditions, suggesting that they may be suitable targets for development of peripherally restricted pain medications.

Disclosures: **M.M. Pando:** None. **T.A. Chavera:** None. **W.P. Clarke:** None. **K.A. Berg:** None.

Poster

708. Pain: Opioid Receptor Pharmacology and Signaling Mechanisms

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 708.01/DD17

Topic: D.02. Somatosensation: Pain

Support: NIH grant NS073935

Title: Role of inhibitory g proteins in opioid-induced hyperalgesia and tolerance

Authors: ***L. LI**, S.-R. CHEN, H.-L. PAN;
MD Anderson Cancer Ctr., Houston, TX

Abstract: Opioids are commonly used to treat patients with moderate and severe pain. However, opioid-induced hyperalgesia and tolerance (OHT) largely limit the opioid efficacy and lead to opioid dose escalation. Although the analgesic action of opioids is mediated by inhibitory Gαi/o-type G proteins, it is unclear whether Gαi/o proteins at the spinal cord level play a role in OHT development. To study the role of individual Gαi/o proteins at the spinal cord level in OHT, we used intrathecal injection of siRNAs targeting specific Gαi/o proteins in rats. Morphine was injected systemically, and nociception was tested with a radiant heat and a noxious pressure stimuli applied to the hindpaw. We found that treatment with Gαi1-specific and Gαi3-specific siRNA did not significantly affect the morphine analgesic effect and OHT development. In contrast, intrathecal treatment with Gαi2-specific or Gαo-specific siRNA significantly diminished the acute analgesic effect of morphine. Mechanical and thermal hyperalgesia and the reduction in the analgesic effect after 5 days of morphine treatment were significantly greater in Gαi2 siRNA-treated rats than in control siRNA-treated rats. Gαo knockdown unexpectedly increased the baseline heat, but not mechanical, nociceptive threshold following daily morphine treatment. Furthermore, the analgesic effect of morphine was completely lost after 5 days of treatment in Gαo siRNA-treated rats. These results suggest that Gαi2 and Gαo at the spinal cord level play a critical role in mediating the opioid analgesic effect. Gαi2 is also involved in attenuation of OHT development. Supported by NIH grant NS073935.

Disclosures: **L. Li:** None. **S. Chen:** None. **H. Pan:** None.

Poster

708. Pain: Opioid Receptor Pharmacology and Signaling Mechanisms

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Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 708.02/EE1

Topic: D.02. Somatosensation: Pain

Support: University of Arizona Startup Funds

Title: Development of selective peptide antagonists to the mu-delta opioid receptor heterodimer

Authors: K. OLSON[1], V. J. HRUBY[2], *J. M. STREICHER[3];
[1]Pharmacology; Chem. and Biochem., [2]Chem. and Biochem., [3]Pharmacol., Univ. of Arizona, Tucson, AZ

Abstract: The opioid receptors are a family of G protein coupled receptors crucial for the neuromodulation of important brain states including pain, reward, memory, and food intake. While the family is composed of three main members, mu (MOR), delta (DOR), and kappa, it has long been appreciated from pharmacological and other experiments that more than three

forms of these receptors likely exist. These identified forms include splice variants as well as heterodimerization between the opioid receptor family members as well as with non-opioid receptors. One such identified heterodimer is the mu/delta (M/DOR) heteromer. Using in vitro models, the heterodimerization binding site of the M/DOR has been identified, and it has been shown that the M/DOR invokes different signal transduction cascades than either monomer. Recently an M/DOR selective antibody was developed, which demonstrated that the M/DOR is upregulated in the brains of chronic morphine treated mice, and may mediate opioid tolerance, among other potential effects. Despite these advances however, there are very few tools to selectively target the M/DOR, and no selective antagonists that we could find, making it difficult to determine the role of the M/DOR in opioid and pain physiology. To address this lack we synthesized a series of potential selective peptidic M/DOR antagonists by coupling two moderate to low affinity MOR and DOR antagonist pharmacophores with a flexible and variable-length linker (15-42 atoms in the initial series), hypothesizing that we could attain high selectivity for the M/DOR by using two moderate/low affinity pharmacophores that would have an avidity induced improvement in M/DOR potency. We then characterized this antagonist series in vitro using competition radioligand binding and ^{35}S-GTPγS coupling in antagonist mode using CHO cells expressing the MOR or DOR homomers, or the M/DOR heteromer. Our initial results demonstrate a linear relationship between the linker length and the antagonist potency at the M/DOR, with the 15 atom linker compound having the best potency to date (5.1 nM, 5.1-187 nM range). We also demonstrate at least a 30 fold selectivity with the 15 atom linker compound between the M/DOR and either the MOR or DOR homomer, validating our strategy. Beyond these initial results, we will also explore shorter linker lengths, alternate pharmacophores, and varying the rigidity of the linker. After completion of in vitro characterization and the selection of the highest selectivity and potency M/DOR antagonist, we will explore the ability of our antagonist to reverse chronic morphine induced tolerance in vivo, among other potential effects of the M/DOR in vivo.

Disclosures: **K. Olson:** None. **V.J. Hruby:** None. **J.M. Streicher:** None.

Poster

708. Pain: Opioid Receptor Pharmacology and Signaling Mechanisms

Location: Halls B-H

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Program#/Poster#: 708.03/EE2

Topic: D.02. Somatosensation: Pain

Support: Quebec Pain Research Network

CIHR grant MOP-123399

Title: Knockin of delta opioid receptors in primary afferents alleviates heat-induced pain

Authors: *K. ABDALLAH[1], V. BLAIS[2], K. BRADBURY[4], K. FONTES[4], J.-L. PARENT[3], C. CAHILL[5], J. BOULTER[6], L. GENDRON[2];
[1]Dept. of Pharmacol. and Physiol., Univ. of Sherbrooke, Sherbrooke, QC, Canada; [2]Dept. of Pharmacol. and Physiol., [3]Dept. of Med., Univ. de Sherbrooke, Sherbrooke, QC, Canada; [4]Bishop's Univ., sherbrooke, QC, Canada; [5]Dept. of Anesthesiol. and perioperative care school of medicine, Univ. of California-Irvine, Irvine, CA; [6]Dept. of Psychiatry & Biobehavioral Sci., Univ. of California, Los Angeles, CA

Abstract: Due to their limited adverse effects, delta opioid receptors (DOPrs) represent a promising target for the treatment of chronic pain and emotional disorders. Although DOPr agonists produce only weak analgesic effects in healthy animals and in acute pain models, we and others have previously shown an increased in their potency to relieving chronic pain (e.g. inflammatory, neuropathic, and bone cancer-induced pain). Interestingly, we observed that this increase in the analgesic effects of DOPr agonists is paralleled by a translocation of DOPr from the intracellular compartment to the plasma membrane. In order to study the roles of DOPr *in vivo* and to study the molecular and cellular mechanisms regulating its trafficking, we have generated a genetically-engineered mouse in which a Flag epitope has been introduced between the first and second amino acids of DOPr. A floxed translational stop cassette was also introduced upstream of the initiation codon. In the absence of recombination, these mice (STOP::Flag-DOPr) behave as DOPr knockout mice. We first bred the STOP::Flag-DOPr mice with Zp3-Cre mice to generate mice (Flag-DOPr knockin) expressing a Flag-tagged DOPr in place of the endogenous receptor. A thorough characterization of these mice reveal that the Flagged receptor is expressed at similar levels and in the same areas as its endogenous counterpart. We also observed that DOPr agonists produces similar behavioral effects in the Flag-DOPr knockin mice and in wildtype animals. Therefore, these mice display pharmacological and behavioral properties similar to mice expressing endogenous DOPr. In the human lumbar spinal cord, DOPr is primarily expressed in the terminals of primary afferents. To specifically study the role of DOPr in these neurons, we infected newborn STOP::Flag-DOPr mice with a rAAV2/9-CBA-Cre-GFP adenovirus. The virus was injected at P5 in the hindpaws of the animals. Two months after the injection, GFP is expressed in the nuclei of approximately 60% of the lumbar dorsal root ganglia neurons. No GFP staining was observed in the spinal cord nor in the brain, indicating that the infection was restricted to the primary afferent. When these mice were injected with the Complete Freund's adjuvant in one hindpaw, we found that the intrathecal administration of deltorphin II produces analgesia in the thermal plantar test, indicating that presynaptic DOPr are able to inhibit heat-induced pain. These results are important since they support a role for DOPr-expressing nociceptors in heat-induced pain, a dogma that was recently challenged.

Disclosures: K. Abdallah: None. **V. Blais:** None. **K. Bradbury:** None. **K. Fontes:** None. **J. Parent:** None. **C. Cahill:** None. **J. Boulter:** None. **L. Gendron:** None.

Poster

708. Pain: Opioid Receptor Pharmacology and Signaling Mechanisms

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Program#/Poster#: 708.04/EE3

Topic: D.02. Somatosensation: Pain

Support: (National Institute of Drug Addiction, grant #05010

National Institute on Alcohol Abuse and Alcoholism, grant #16658

Title: Bold imaging in awake wild-type and mu-opioid receptor knock-out mice reveals on-target activation maps in response to oxycodone

Authors: *K. MOORE[1], D. MADULARU[2], S. IRIAH[1], J. YEE[1], P. KULKARNI[1], E. DARCQ[2], B. KIEFFER[2], C. FERRIS[1];
[1]Psychology, Northeastern Univ., Boston, MA; [2]Douglas Hosp. Res. Inst., McGill Univ., Montreal, QC, Canada

Abstract: Blood oxygen level dependent (BOLD) imaging in awake mice was used to identify differences in brain activity between wild-type, and Mu (μ) opioid receptor knock-outs (MuKO) in response to oxycodone (OXY). Using a segmented, annotated MRI mouse atlas and computational analysis, patterns of integrated positive and negative BOLD activity were identified across 122 brain areas. The pattern of positive BOLD showed enhanced activation across the brain in WT mice within 15 min of intraperitoneal administration of 2.5 mg of OXY. BOLD activation was detected in 72 regions out of 122, and was most prominent in areas of high μ opioid receptor density (thalamus, ventral tegmental area, substantia nigra, caudate putamen, basal amygdala and hypothalamus), and focus on pain circuits indicated strong activation in major pain processing centers (central amygdala, solitary tract, parabrachial area, insular cortex, gigantocellularis area, ventral thalamus primary sensory cortex and prelimbic cortex). Importantly, the OXY-induced positive BOLD was eliminated in MuKO mice in most regions, with few exceptions (some cerebellar nuclei, CA3 of the hippocampus, medial amygdala and preoptic areas). This result indicates that most effects of OXY on positive BOLD are mediated by the μ opioid receptor (on-target effects). OXY also caused an increase in negative BOLD in WT mice in few regions (16 out of 122) and, unlike the positive BOLD response the negative BOLD was only partially eliminated in the MuKO mice (cerebellum), and in some case intensified (hippocampus). Negative BOLD analysis therefore shows activation and deactivation events in the absence of the μ receptor for some areas where receptor expression is normally extremely low or absent (off-target effects). Together, our approach permits establishing opioid-induced BOLD activation maps in awake mice. In addition, comparison of WT and MuKO mutant mice reveals both on-target and off-target activation events, and set an OXY brain signature that should, in the future, be compared to other μ opioid agonists.

Disclosures: **K. Moore:** None. **D. Madularu:** None. **S. Iriah:** None. **J. Yee:** None. **P. Kulkarni:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); EKAM. **E. Darcq:** None. **B. Kieffer:** None. **C. Ferris:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); EKAM, Animal Imaging Research.

Poster

708. Pain: Opioid Receptor Pharmacology and Signaling Mechanisms

Location: Halls B-H

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Program#/Poster#: 708.05/EE4

Topic: D.02. Somatosensation: Pain

Support: State of Washington Initiative Measure No. 171

Title: Inhibition of regulator of G-protein signaling proteins enhances morphine but not fentanyl antinociception in the rat.

Authors: *A. T. TRAN[1], S. M. SCHOO[2], M. M. MORGAN[2];
[2]Psychology, [1]Washington State Univ., Vancouver, WA

Abstract: Although opioids are the most effective treatment for chronic pain, their use is hindered by many negative effects (e.g. analgesic tolerance, dependence, respiratory depression). An important goal is to develop treatments that enhance opioid analgesia while diminishing side effects. Opioid antinociception is mediated through the mu-opioid receptor (MOR), a seven-transmembrane G-protein coupled receptor (GPCR). Regulator of G-protein signaling (RGS) proteins interact with the Gα-subunit of GPCRs and catalyzes the hydrolysis of GTP to GDP, rapidly terminating G-protein signaling. We hypothesize that the inhibition of RGS proteins will prolong G-protein signaling and enhance opioid-mediated antinociception. This hypothesis was tested by microinjecting an opioid (morphine or fentanyl) simultaneously with the small molecule RGS inhibitor CCG-63802 into the ventrolateral periaqueductal gray (PAG) of awake rats. The PAG is a midbrain structure known to contribute to opioid antinociception. Both morphine and fentanyl produced dose-dependent antinociception when microinjected into the ventrolateral PAG. Administration of CCG-63802 enhanced morphine potency as indicated by a leftward shift in the dose response curve compared to control animals, but had no effect on fentanyl antinociception. This selective enhancement of morphine but not fentanyl antinociception is consistent with other ligand biased signaling at the MOR and indicates that morphine, but not fentanyl antinociception is mediated by G-protein signaling.

Disclosures: **A.T. Tran:** None. **S.M. Schoo:** None. **M.M. Morgan:** None.

Poster

708. Pain: Opioid Receptor Pharmacology and Signaling Mechanisms

Location: Halls B-H

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Program#/Poster#: 708.06/EE5

Topic: D.02. Somatosensation: Pain

Support: Texas A&M Genomics Seed Grant; Texas A&M University Vice President for Research, Health Science Center, and AgriLife Research

Title: Oxycodone, hydrocodone, and morphine differentially affect gene expression

Authors: ***C. T. HORRAX**[1], M. A. EMERY[1], M. L. S. BATES[1], P. J. WELLMAN[2], S. EITAN[2];
[1]Neurosci., [2]Psychology, Texas A&M, College Station, TX

Abstract: Opioid drugs remain the gold standard in pain treatment, and are regularly prescribed. However, the chronic use of these medications is known to lead to complications such as the development of tolerance, hyperalgesia, allodynia, dependence, and addiction. Additionally, the use of opioids, both medical and nonmedical, has been shown to increase the risk of developing comorbid mental disorders such as anxiety, depression, and alcoholism. This implies a shared mechanism underlying these disorders, and indeed recent research has revealed that the D2DR plays a role in all three. Recent research revealed that opioids disturb the signaling of the D2DRs, which may partially account for this association. Importantly, we recently observed that various opioids differentially modulate the responses of the D2DRs. Specifically, at equianalgesic doses, various opioids have differential effects on the responses of D2DRs to dopamine agonists. Moreover, at equianalgesic doses, various opioids have differential effects on the activity of signaling molecules. Additionally, these drugs have been shown to differ in their efficacy in the treatment of both acute and chronic burn pain. Thus, in the current study we examined the differential effects of oxycodone, hydrocodone, and morphine on striatal gene expression. Specifically, mice were administered with saline or the various opioids for 6 days. Twenty four hours after the final injection, striatal tissue was dissected. Following this, RNA was extracted and high throughput next generation sequencing was used to examine the mouse genome. Subsequently, we used RT-PCR to confirm the results for selected candidate genes. In mice given morphine, the expression of 79 genes was dysregulated, 33 of which were unique to morphine, 15 of which were shared with oxycodone, 9 of which were shared with hydrocodone, and 22 of which were shared with both oxycodone and hydrocodone. In mice given hydrocodone, 71 genes were dysregulated, 27 of which were unique to hydrocodone, and 13 of

which were shared with oxycodone. Most surprisingly, in mice given oxycodone, 179 genes were dysregulated, 158 of which were unique to oxycodone. These data provide strong evidence that there are differences between opioid drugs with regard to their effects on opioid receptors, D2DRs, intercellular signaling, and the dopamine reward system. This information should be considered by physicians when deciding which opioid medication is most efficacious in specific clinical scenarios, and least likely to have harmful effects.

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Poster

708. Pain: Opioid Receptor Pharmacology and Signaling Mechanisms

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 708.07/EE6

Topic: D.02. Somatosensation: Pain

Title: A minimally brain-penetrant, biased μ-opioid receptor (MOR) agonist that is an effective analgesic with a reduced side effect profile

Authors: *S. M. GOEBEL-GOODY[1], A. PIKE[2], S. HUMPHREYS[3], R. TORELLA[4], E. DUNN-SIMS[1], C. HSU[1], C. TYSZKIEWICZ[1], N. NAWREEN[1], D. HORTON[1], R. SUZUKI[3]; [1]Global Safety Pharmacol., Pfizer Inc., Groton, CT; [2]Pharmacokinetics Dynamics & Metabolism, [3]Neurosci. & Pain Res. Unit, [4]Worldwide Medicinal Chem., Pfizer Inc., Cambridge, United Kingdom

Abstract: Conventional μ-opioid receptor (MOR) agonists, such as morphine, oxycodone, and fentanyl, are among the most efficacious and widely-prescribed analgesics for moderate to severe pain. Despite their robust efficacy, they elicit a variety of prohibitive adverse side effects including constipation, nausea, respiratory depression, and sedation. They also induce a state of euphoria and physical dependence that can lead to misuse or abuse. MORs are G protein-coupled receptors (GPCRs), where activation is primarily mediated via dissociation of the G protein complex and production of downstream signaling molecules, such as cAMP. On the other hand, desensitization of MORs upon ligand binding is mediated by β-arrestin recruitment and subsequently clathrin-mediated endocytosis. Converging lines of evidence have demonstrated that at least some of the side effects associated with MOR agonists may be mediated by the β-arrestin pathway. The objective of these studies was to investigate a structurally novel biased MOR agonist with minimal brain penetration, Compound A. Compound A was administered subcutaneously and evaluated in male Sprague Dawley rats in the acetic acid-induced writhing pain model to confirm analgesic efficacy and in the charcoal transit model to assess

gastrointestinal (GI) function. Given that the subjective or perceived effects of a drug can greatly influence propensity for abuse, Compound A was also assessed in a two-lever food-reinforced drug discrimination procedure to determine if it shares similar discriminative stimulus effects with morphine. Compound A induced a dose-dependent decrease in the number of writhes induced by acetic acid, with >90% efficacy achieved. Doses for the charcoal transit and drug discrimination studies were selected to target efficacious exposures achieved in the acetic acid-induced writhing model. The effect of Compound A on GI motility was less than that of morphine at similar levels of analgesic efficacy. Furthermore, administration of Compound A produced an increase in morphine-appropriate responding that resulted in only a partial generalization to the morphine cue when compared with vehicle. Taken together, these data support the hypothesis that a biased MOR agonist is an effective analgesic with reduced effects on GI function and yet still shares some, but not complete overlap in the discriminative stimulus properties with morphine, despite minimal brain penetration.

Disclosures: **S.M. Goebel-Goody:** A. Employment/Salary (full or part-time): Pfizer, Inc. **A. Pike:** A. Employment/Salary (full or part-time): Pfizer, Inc. **S. Humphreys:** A. Employment/Salary (full or part-time): Pfizer, Inc. **R. Torella:** A. Employment/Salary (full or part-time): Pfizer, Inc. **E. Dunn-Sims:** A. Employment/Salary (full or part-time): Pfizer, Inc. **C. Hsu:** A. Employment/Salary (full or part-time): Pfizer, Inc. **C. Tyszkiewicz:** A. Employment/Salary (full or part-time): Pfizer, Inc. **N. Nawreen:** A. Employment/Salary (full or part-time): Pfizer, Inc. **D. Horton:** A. Employment/Salary (full or part-time): Pfizer, Inc. **R. Suzuki:** A. Employment/Salary (full or part-time): Pfizer, Inc..

Poster

708. Pain: Opioid Receptor Pharmacology and Signaling Mechanisms

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 708.08/EE7

Topic: D.02. Somatosensation: Pain

Title: Morphine-induced analgesia on sensory and affective components of complete freund's adjuvant (CFA)-induced inflammatory pain.

Authors: *A. ARMENDARIZ[1], A. NAZARIAN[2];
[2]Pharmaceut. Sci., [1]Western Univ. of Hlth. Sci., Pomona, CA

Abstract: The experience of pain is characterized by the presence of an aversive sensory stimulus combined with negative affect, which is often mediated clinically through administration of analgesics such as morphine or other prescribed opioids. Although an extensive body of work has elucidated much of the physiological mechanisms involved in the sensory

component of pain, many questions regarding the affective component of pain remain elusive. The present study investigates the effects of morphine on sensory and affective components of pain following administration of Complete Freund's Adjuvant (CFA), a model of inflammatory pain. An intraplantar injection of CFA was administered into the left hind paw of male and female Sprague-Dawley rats. Hargreaves tests for thermal nociception and conditioned place preference (CPP) data were obtained following subcutaneous administration of varying morphine doses (0, 1, 4, 8 mg/kg). Hargreaves Test results revealed sex differences in paw withdrawal latencies (PWL) in a dose dependent manner, with females being less sensitive to morphine than males. CPP results revealed sex differences in the preference for the morphine-paired chamber after one-day post-CFA, but not seven days post-CFA. These results reveal sexually dimorphic properties of morphine analgesia on the affective and sensory components of pain. Considering these findings, further investigation into the underlying mechanisms of morphine analgesia in the affective and sensory component of inflammatory pain is needed.

Disclosures: A. Armendariz: None. **A. Nazarian:** None.

Poster

708. Pain: Opioid Receptor Pharmacology and Signaling Mechanisms

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Program#/Poster#: 708.09/EE8

Topic: D.02. Somatosensation: Pain

Support: Fundación Alfonso Martín Escudero

Title: Cb_1 cannabinoid and delta opiod receptor heteromers in neuropathic pain

Authors: *S. SIERRA SAN NICOLAS[1], A. GUPTA[2], I. GOMES[2], L. DEVI[2];
[1]Icahn Sch. of Med. At Mount Sinai, New York City, NY; [2]Dept. of Pharmacol. Systems, Icahn Sch. of Med. at Mount Sinai, New York City, NY

Abstract: Opioid and cannabinoid receptor function is modulated by multiple mechanisms and both receptors are critical to a number of physiological processes in health and disease including nociception. We have previously reported that CB_1 and delta receptors associate to form heteromers that exhibit distinct pharmacological properties and disease-specific dysregulation including during neuropathic pain. Hence these receptors have been posited as desired targets for pain relief; however, the in vivo consequences of heteromer signaling or their specific location along the pain circuit is not fully explored. Towards this end, we have initiated studies using the cancer chemotherapy drug paclitaxel-induced allodynia (mechanical allodynia) as a model of neuropathic pain. Mice (C57BL/6J mice; 10/group) were treated with vehicle or paclitaxel on

alternate days (cumulative doses 16 mg/kg); paclitaxel-induced (mechanical) allodynia was assessed on days 0, 4, 7 and 15. On day 16, tissues were collected and levels of CB_1 and delta receptors in spinal cord and brain regions were assessed using a receptor-selective or heteromer-selective antibodies and proximity based techniques. We find a significant increase in the abundance of CB_1-delta receptor heteromers in the spinal cord and basolateral amygdala of animals with allydonia. There were no changes in heteromer levels in a number of other regions including cortex, nucleus accumbens, hippocampus, or periaqueductal gray. Studies to characterize the cell types that show increased heteromer levels and to delineate the differential signaling pathway driven by this heteromer are underway. Together, these results suggest an important role for pain in orchestrating the regulation of CB1-DOR heteromer levels and signaling in specific pain related pathways. This work was supported by NIH grants DA008863 and NS026880 to L.A.D and Fundación Alfonso Martín Escudero to S.S.

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Poster

708. Pain: Opioid Receptor Pharmacology and Signaling Mechanisms

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Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 708.10/EE9

Topic: D.02. Somatosensation: Pain

Support: NIDA R01 DA015438

NIDA T32 DA007097-32

Title: Peripherally restricted opioid combination therapy synergizes in multiple pain states

Authors: *D. J. BRUCE[1], C. D. PETERSON[2], K. F. KITTO[3], C. A. FAIRBANKS[4], G. L. WILCOX[5];
[1]Pharmacol., [2]Exptl. and Clin. Pharmacol., [3]Neurosci., [4]Neurosci., Pharmacol., Exptl. and Clin. Pharmacology, Pharmaceutics, [5]Neurosci., Pharmacol., Dermatol., Univ. of Minnesota, Minneapolis, MN

Abstract: Background: The adverse effects of opioid pharmacotherapy are largely mediated by the activation of mu-opioid receptors (MORs) in the central nervous system. However, opioid receptors also mediate analgesia on the peripheral terminals of primary sensory afferents. Despite more than a decade of research directed at developing peripherally restricted opioids, no opioid analgesic to date has delivered pain control without the risk for addiction and diversion. We recently published that morphine and agonists at delta-opioid receptors (DORs) synergized

in spinal cord, and that this interaction was dependent on Protein Kinase C epsilon (PKCε). We interpreted this finding to mean that the synergy occurs on the central terminal of primary afferents, and reasoned that this MOR-DOR synergy should generalize to the peripheral terminals of those same nociceptors. For this study, we chose loperamide (Lo), a highly efficacious MOR agonist that is excluded from the CNS, and oxymorphindole (OMI), a DOR agonist that was shown to synergize with morphine spinally.
Methods: Using the Hargreaves and tail flick thermal nociception assays, we evaluated the analgesic effect of loperamide, OMI, or their 1:1 combination in naive mice. Drugs were delivered by spinal (intrathecal, i.t.), local (intraplantar, i.pl.) or systemic (subcutaneous, s.c.) injections. We also examined the anti-hyperalgesic effect of the drugs or their combination in the mouse model of Freund's Complete Adjuvant (FCA)-induced chronic inflammatory pain (subjects injected i.pl. with FCA 3-6 days earlier). Finally, the efficacy of a transdermal formulation of OMI-Lo was tested in the FCA model by applying drugs in an ethanol solution directly onto the hindpaw.
Results: Our initial efficacy studies show that when combined in a 1:1 dose ratio—either locally or systemically—OMI-Lo produces analgesia (naïve subjects) at 4- to 10-fold lower doses or anti-hyperalgesia (inflamed subjects) at 50- to 100-fold lower doses than either agent given alone. This synergy is blocked by the peripherally restricted opioid antagonist, naloxone methiodide, reinforcing the peripheral localization of the effect. Lastly, OMI-Lo is able to produce potent anti-hyperalgesia when delivered as a transdermal solution.
Conclusions: MOR agonists significantly synergize with DOR agonists at peripheral sites of action, providing strong evidence in support of peripherally restricted combination therapy. The systemic and transdermal efficacy of the combination, along with loperamide's extremely low abuse liability, suggests that this combination therapy might be therapeutically useful to control inflammatory pain in the clinic.

Disclosures: D.J. Bruce: None. **C.D. Peterson:** None. **K.F. Kitto:** None. **C.A. Fairbanks:** None. **G.L. Wilcox:** None.

Poster

708. Pain: Opioid Receptor Pharmacology and Signaling Mechanisms

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 708.11/EE10

Topic: D.02. Somatosensation: Pain

Support: State of Washington Initiative Measure No. 171

Title: Home cage wheel running as a method to assess morphine side effects, tolerance, and withdrawal

Authors: *J. J. CALSBEEK[1], A. T. LEE[1], R. KANDASAMY[2], M. M. MORGAN[1]; [1]Psychology, Washington State Univ. Vancouver, Vancouver, WA; [2]Integrative Physiol. and Neurosci., Washington State Univ., Vancouver, WA

Abstract: Although opioids are the most effective treatment for pain, the use of opioids is limited because of unpleasant side effects and the development of tolerance and dependence. We recently have shown that home cage wheel running is an effective method to assess nociception (Kandasamy et al., 2016) and may be a useful way to assess opioid side effects, tolerance, and dependence. We tested this hypothesis by measuring home cage wheel running before and following repeated injections of morphine into male Sprague-Dawley rats. Rats were individually housed in a cage with a running wheel for 7 days prior to morphine (5 mg/kg, s.c.) or saline administration. Rats received two more injections on each of the next three days. Naltrexone (2 mg/kg, s.c.) or saline was administered 30 min after the last morphine/saline injection to precipitate withdrawal. Administration of morphine caused a complete cessation of wheel running that recovered to baseline levels by the third hour after the injection. Tolerance was evident to morphine-depressed wheel running as was evident by a reduction in the magnitude of depressed wheel running on subsequent days. Administration of naltrexone following the seventh morphine injection induced mild signs of physiological withdrawal and produced a significant decrease in wheel running that persisted for over 2 hrs. Administration of naltrexone also depressed wheel running in control rats not given morphine, but this decrease was only evident for the first hour after the injection. These data indicate that home cage wheel running is a useful method to assess opioid side effects and withdrawal in the rat.

Disclosures: J.J. Calsbeek: None. **A.T. Lee:** None. **R. Kandasamy:** None. **M.M. Morgan:** None.

Poster

708. Pain: Opioid Receptor Pharmacology and Signaling Mechanisms

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 708.12/EE11

Topic: D.02. Somatosensation: Pain

Support: NHMRC

Title: Mu-opioid receptor signaling mechanisms: role of kinetics in biased agonism

Authors: *S. SIANATI[1], A. YOUSUF[1], M. CHRISTIE[1], M. CANALS[2];
[1]Sydney Univ., Sydney, Australia; [2]Pharm. and pharmaceutical science, Monash, Melbourne, Australia

Abstract: Opioid analgesics are the most efficacious drugs for treatment of acute and chronic pain and hold the major market share of pain medications. However, the clinical utility of opioids is limited by development of tolerance and physical dependence during long-term administration. Improving the effectiveness and safety profile of opioids have remained major goals in drug discovery.
The notion of biased signaling offers a new perspective as a means to separate therapeutic effects from unwanted side effects. This concept refers to the ability of different agonists to distinctively activate different signaling pathways while binding to the same receptor. Recent studies have indicated that G-protein versus βarrestin (and endocytosis) bias influences opioid tolerance and other side effects of opioids; suggesting bias towards G-protein improves safety profile. However the molecular basis underlying bias is not resolved yet.
The aim of this study was to determine the mechanisms behind bias at MOR by considering the kinetic properties of agonist. Efficacy, bias and kinetics were determined in G-protein signal, Ser^{375} phosphorylation, βarr2 recruitment and internalization (signaling cascades triggered by activated MOR) for a series of widely used, potent opioids having a large range of G-protein versus endocytosis bias. The present study illustrated that opioid kinetic is agonist dependent. Inactivation of each pathway depends on ligand dissociation (residence time) rather than biochemical kinetics. Off-rate kinetics for G-protein were highly correlated with Ser^{375} and βarr2 off-rates (r_{xy} = 0.96 and 0.91 respectively). Most importantly, biased efficacies were strongly correlated with the off-rate kinetics for each pathway (r_{xy} = 0.93, 0.96 and 0.98 for G-protein, Ser^{375} and βarr2 off-rate respectively). These results indicating that bias towards endocytosis at opioids requires sustained agonist occupancy. Slowly dissociating agonists such as endomorphins with long residence time develop endocytosis versus G-protein bias, suggesting for the first time that the residence time of an agonist at MOPr controls bias.

Disclosures: S. Sianati: None. **A. Yousuf:** None. **M. Christie:** None. **M. Canals:** None.

Poster

708. Pain: Opioid Receptor Pharmacology and Signaling Mechanisms

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 708.13/EE12

Topic: D.02. Somatosensation: Pain

Support: CIHR Grant MOP273137

Quebec Pain Research Network

Title: Constitutive internalization of delta opioid receptors

Authors: *S. GRASTILLEUR[1], J. SIMARD[1], J.-L. PARENT[2], C. LAVOIE[1], L. GENDRON[1];
[1]Pharmacologie-Physiologie, [2]Médecine, Univ. De Sherbrooke, Sherbrooke, QC, Canada

Abstract: Agonist-induced activation of G protein coupled receptors (GPCRs) produces their internalization followed by their recycling or degradation. In addition to the agonist-induced internalization, the delta opioid receptor (DOPr) was shown to be constitutively internalized through a clathrin-dependent pathway. However, to date the characterization of this mechanism is poorly described in the literature. Here, we used the proximity-dependent biotin identification (Bio-ID) coupled to mass-spectrometry to identify novel putative DOPr interacting partners. The biotin ligase Bir A* was fused to the carboxy-terminal tail of the rat DOPr and transfected in NG108-15 cells. Following incubation of cells stably expressing DOPr-Bir A* with biotin, the biotinylated proteins were isolated using sepharose-streptavidin beads then identified by mass spectrometry analysis.
193 of 1557 proteins were considered as being specific and potentially interacting with DOPr. These proteins belong to 21 different families (examples are cell adhesion molecules, membrane receptors, transporters, trafficking, chaperones, and signalling molecules). Some proteins of the endocytic pathway have also been identified (including AP-2, clathrin heavy chain 1, clathrin interactor 1, various Rab). Immunofluorescence experiments performed on stable HEK cells expressing Flag-DOPr revealed that the constitutive internalization of DOPr was Rab5-dependent. Most interestingly, we failed to observe a significant accumulation of DOPr in the lysosomes in the presence of chloroquine, suggesting that the agonist-independent internalized DOPr is primarily recycled back to the plasma membrane. Whether constitutively internalized DOPr is trafficked via the fast recycling pathway (Rab4 dependent) or the slow recycling pathway (Rab11 dependent) remains to be determined.
Regulating the level of constitutive internalization of DOPr represents an opportunity to control the level of DOPr expressed at the cell surface and, by way of consequence, the level of analgesia induced by delta agonists. Therefore, a better knowledge of the mechanisms involved in the regulation of DOPr trafficking may lead to the development of new strategies to increase the analgesic potency of delta agonists.

Disclosures: S. Grastilleur: None. **J. Simard:** None. **J. Parent:** None. **C. Lavoie:** None. **L. Gendron:** None.

Poster

708. Pain: Opioid Receptor Pharmacology and Signaling Mechanisms

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 708.14/EE13

Topic: D.02. Somatosensation: Pain

Support: Shirley and Stefan Hatos Foundation (CMC, SL)

Canadian Institutes of Health Research (LX, MCO) - MOP123298

Title: Kappa opioid receptor activation in neuropathic pain regulates reward

Authors: S. LIU[1], L. XUE[3], M. C. OLMSTEAD[3], F. M. LESLIE[1], F. I. CARROLL[4], *C. M. CAHILL[2];
[1]Pharmacol., [2]Anesthesiol. & Perioperative Care, Univ. of California Irvine, Irvine, CA;
[3]Queen's Univ., Kingston, ON, Canada; [4]Res. Triangle Inst. Intl., Research Triangle Park, NC

Abstract: The kappa opioid receptor (KOR) is known to regulate mood, motivation and reward pathways in the brain. It has been shown that activation of KORs at the nucleus accumbens (NAc) leads to a decrease in dopamine neurotransmission. Antagonists for this receptor have anxiolytic and anti-depressant effects in preclinical animal models, which are possibly due to the inhibitory tone of KOR on dopamine release. Chronic pain leads to anxiety and depression in patients, which negatively impacts their quality of life. Previous studies in acute pain models have determined that KOR activation does not alter anxiety and depression; however, it is unknown if KOR function alters motivation or negative affect in chronic pain states. It is hypothesized that chronic neuropathic (NP) pain leads to a decreased dopaminergic tone within the mesocorticolimbic pathway. In this study, we aimed to determine whether KOR activity is increased in NP pain and whether blockade of this receptor recovers reward in this chronic pain state. We induced NP pain in adult male C57/BL6 mice or Long Evans rats by implanting a polyethylene cuff around their sciatic nerve of the left hind limb. Using [35S]-GTPγS autoradiography, we found that NP pain mice exhibited significant increases KOR activation and/or availability in the NAc and ventral tegmental area (VTA) when compared to sham surgical controls. Conversely, we observed decreased MOR activation in the VTA of NP pain mice, suggesting either receptor down-regulation or desensitization. Previous studies in our lab identified that NP pain caused a blunting of reward circuitry where intra-VTA injection of MOR agonist DAMGO failed to produce a place preference. These data can be explained by a decrease in MOR function (possibly desensitization) as evidenced by [35S]-GTPγS autoradiography data. However, because KOR system is upregulated in NP pain conditions, we asked whether blockade of KOR could restore DAMGO-induced reward in NP pain. Pretreatment of the KOR antagonist JDTic prior to conditioning with intra-VTA DAMGO had no effect on place preference in sham animals but restored reward in NP pain animals. These results demonstrate

that NP pain increases the expression and activation of KOR as well as a corresponding decrease in the expression and function of MORs in the dopaminergic reward pathway. Long-term KOR signaling may be regulating the functional effects of MORs in reward. This interaction provides insight to understanding why mu-opioid-based therapeutics to treat chronic pain in the clinic become less effective over time. This study also confirms the importance of KOR function with regard to reward in the chronic pain state.

Disclosures: **S. Liu:** None. **L. Xue:** None. **M.C. Olmstead:** None. **F.M. Leslie:** None. **F.I. Carroll:** None. **C.M. Cahill:** None.

Poster

708. Pain: Opioid Receptor Pharmacology and Signaling Mechanisms

Location: Halls B-H

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Program#/Poster#: 708.15/EE14

Topic: D.02. Somatosensation: Pain

Support: NIH Intramural Support

Title: Knockout of r7bp suppresses somatosensory itch perception by enhancing kappa opioid mediated inhibition

Authors: ***M. PANDEY**[1], J.-H. ZHANG[1], P. ADIKARAM[1], W. SIMONDS[1], M. HOON[2], S. MISHRA[3], R. NEUBIG[4];
[1]Metabolic Dis. Br., NIH, NIDDK, Bethesda, MD; [2]Lab. of Sensory Biol., Natl. Inst. of Dent. and Craniofacial Res., Bethesda, MD; [3]Lab. Of Sensory Biol., (National Inst. of Dent. and Craniofacial Res., Bethesda, MD; [4]Dept. of Pharmacol. and Toxicology, Univ. Michigan, Ann Arbor, MI

Abstract: R7-binding protein (R7bp) facilitates the G i//o-directed GTPase activating protein (GAP) activity of complexes containing Gbeta5 and a regulator of G protein signaling protein belonging to the R7 subfamily (R7-RGS), thereby accelerating the termination of Gi/o signaling. We discovered that mice lacking R7bp have an abnormal somatosensory phenotype manifest by diminished scratching responses to a variety of pruritogens administered intradermally or intrathecally. In contrast, most of the nociceptive modalities tested were not different in R7BP knockout (KO) mice as compared to wild type. The pruriceptive defect in *R7bp* KO mice was abolished in double KO mice also lacking *Oprk1*, encoding the G protein-coupled kappa opioid receptor whose activation inhibits itch sensation. The anti-pruritic effect of kappa opioid agonists was potentiated in *R7bp* KO mice. Taken together, our results indicate that R7bp normally enhances pruriception by inhibiting G protein signaling coupled to, or downstream of, the kappa-

opioid receptor and suggest the potential targeting of R7bp-dependent GAP activity as a novel therapeutic strategy for pathological itch.

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Poster

709. Somatosensation: Plasticity

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 709.01/EE15

Topic: D.03. Somatosensation: Touch

Support: MEXT-Supported Program for the Strategic Research Foundation at Private Universities, 2014–2018

Title: Brain plasticity changes by playing wind instruments and piano

Authors: ***Y.-W. SUNG**, D. KANG, Y. KAWACHI, S. OGAWA;
Kansei Fukushi Inst, Tohoku Fukushi Univ., Sendai-Shi, Japan

Abstract: Neuroimaging studies have showed plasticity changes in the human brain by training or learning. For musical training the plasticity change was observed in musical genres such as piano playing, violin playing or singing. However, when one person has experiences playing several musical instruments, differences in the plasticity change between musical instruments is not yet known well. In this study, we compared plasticity changes in the brain by musical training in piano and wind instruments by voxel-based morphometry (VBM) and resting state fMRI (rs-fMRI) signals. Twenty-four college students who were playing wind instruments and also had experiences of piano playing were participated. All subjects were scanned by 3T MRI (Siemens) in two sessions of structural (T1) and functional imaging (resting-state fMRI). The training period (years) for wind instruments showed a positive correlation with VBM data at a brain area, the left superior temporal gyrus (anterior of BA22). But the training period of the piano did not show correlation with any brain area at the same significance level. A seed-based analysis with the postcentral gyrus (Post-CG) as the seed was performed in correlation with the training period for wind instruments and piano respectively. Post-CG had more significant correlation with pre-center gyrus (Pre-CG) for the training period of wind instruments than for that of piano. Another seed-based analysis with STG as the seed revealed that many brain areas had significant correlation between STG and the training periods of wind instruments or piano experiences. The areas shown correlation with the wind instrument training only were the left supramarginal gyrus (SMG), the left frontal pole (FP) and the bilateral paracingulate cortices

(Para-CC). The areas shown correlation with the piano training only were the superior division of the right lateral occipital cortex and caudate nucleus. SMG is part of the somatosensory association cortex, which interprets tactile sensory data. The core function of the FP is known as processing of the cognitive branching that enables multiple tasks. Para-CC is involved in social cognition. These suggest that the training of wind instruments affects brain functions related to somatosensory, multiple tasks and social cognition. The caudate nucleus and lateral occipital cortex are known to be involved in working memory, and the caudate nucleus is also known to be involved in motor processing. These suggest that piano training affects working memory and motor function. Those results, together, indicate that brain plasticity change is related specifically to the playing styles as well as the musical instrument per se.

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Poster

709. Somatosensation: Plasticity

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Program#/Poster#: 709.02/EE16

Topic: D.03. Somatosensation: Touch

Support: Envision Research Institute Postdoctoral Research Grant (ERI-F02-ARK1)

Title: Aesthetic appreciation and perception of 3D shapes through the sense of touch in the blind and the sighted: on the properties of contour curvature and symmetry of 3D tactile shapes

Authors: *A. KARIM[1], L. LIKOVA[2];
[1]Envision Res. Inst., Wichita, KS; [2]The Smith-Kettlewell Eye Res. Inst., San Francisco, CA

Abstract: Whether blind people are different from the sighted in perceiving the world is a long-standing question. Research has shown that sighted people prefer a curved visual object over a sharp one (Bar & Neta, 2007), and a symmetric visual object over an asymmetric one (Shepherd & Bar, 2011). However, it has rarely been studied how people perceive 3D tactile objects, how they characterize them, and whether the appreciation of 3D tactile objects can be affected by early visual experience. In order to fill this gap, we investigated tactile discrimination, and appreciation and preference for 3D tactile shapes in congenitally blind, late-onset blind and blindfolded sighted participants.
Method: 15 congenitally blind, 13 late-onset blind and 11 blindfolded sighted adults were tested in two different experiments. Nine pairs of 3D tactile geometric shapes (sharp vs curved) were presented in Experiment 1, and ten pairs of 3D tactile shapes (symmetric vs asymmetric) were presented in Experiment 2. Participant's task was to explore and compare the members of each

stimulus pair with two hands, and respond to a set of 14 questions (using a 2AFC paradigm) designed to measure their tactile discriminability, tactile preference, and aesthetic appreciation. Results and Discussion: Analysis of data in MANOVA revealed that there was no difference in tactile discrimination (sharpness, symmetry) between the three groups of participants. On average, they exhibited significantly more preference or aesthetic bias for a curved 3D tactile shape over a sharp one and for a symmetric 3D tactile shape over an asymmetric one. These findings are in line with previous findings in the visual modality (Bar & Neta, 2007; Shepherd & Bar, 2011). Though we did not find any overall difference between the groups we did observe that the proportion of individuals making a preference or aesthetic bias for a sharp or asymmetric 3D tactile shape were greater in the blind than the sighted. More interestingly, both the blind and sighted participants characterized the sharp or asymmetric 3D tactile shapes by more emotionally intense attributes as compared to the curved or symmetric shapes. This suggests that sharp or asymmetric 3D tactile shapes may have inherent capacity to produce a greater response in the brain areas engaged in emotion processing as compared to the curved or symmetric shapes. This is partly supported by the finding that sharp visual objects produce an increased activation of the amygdala as compared to the curved visual objects (Bar & Neta, 2007). All these findings are very informative and lead to further research on the underlying brain mechanisms or neural correlates of tactile shape and aesthetic processing.

Disclosures: A. Karim: None. **L. Likova:** None.

Poster

709. Somatosensation: Plasticity

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Topic: D.03. Somatosensation: Touch

Support: NIH grant NS16446 to JHK

NIH grant NS067017 to HXQ

Craig H. Neilsen Foundation fellowship to JLR, JHK

Title: Second-order spinal cord pathway contributes to cortical reactivation in New World monkeys after long recovery times from incomplete dorsal column lesions

Authors: *C.-C. LIAO, H.-X. QI, J. L. REED, J. H. KAAS;
Dept. of Psychology, Vanderbilt Univ., Nashville, TN

Abstract: After long recovery times (> 6 months) from dorsal column lesions (DCLs), rewiring of neuronal connections in the somatosensory system underlies the cortical reactivation of somatosensory areas 3b and 1. The second-order spinal cord projections from below the lesion level could contribute to the functional recovery after DCLs because these neurons convey tactile inputs to the ipsilateral cuneate nucleus (Cu) through the cuneate fasciculus and lateral funiculus. We tested this hypothesis by comparing the distributions of second-order spinal cord neurons in the cervical spinal cord in normal monkeys and monkeys after short and long-term recovery periods after DCLs. Nine New World monkeys (*Saimiri boliviensis*; 5 male and 4 female) were used in this study. In 2 normal monkeys, a retrograde tracer, cholera toxin subunit B (CTB), was injected into the digit representation in the Cu to label the second-order spinal cord neurons. In 7 monkeys, unilateral DCLs were made at the C4 level. After 2 weeks (short-term; n=3) or after 7-9 months (long-term; n=4), CTB was injected into the Cu ipsilateral to the lesion site. We mapped hand representations in the contralateral areas 3b and 1 by microelectrode multiunit recordings to investigate the cortical reactivation after injuries. The labeled neurons in the cervical spinal cord were plotted and quantified. Our results revealed that: 1) in normal monkeys, the labeled neurons were extensively distributed in the cervical spinal cord ipsilateral to the tracer injections site in the Cu. 2) After short recovery periods in monkeys with extensive DCLs (91-100% complete), the hand representations in contralateral areas 3b and 1 remained unresponsive or only weakly responsive to touch on hand. The proportions of labeled spinal cord neurons in the ipsilateral C5-C8 below the lesion (8.3% -14.8%) were greatly decreased compared to the normal monkeys (54.4% and 56.5%). 3) After long recovery times, the deafferented somatosensory cortex of 2 monkeys with complete DCLs responded weakly to touch on face and forelimb, and occasionally on the hand. The proportions of labeled neurons in the ipsilateral spinal cord below the lesion were only 3.6% and 5.0%. In 2 monkeys with large but incomplete DCLs (87% and 72% complete), the hand cortex in areas 3b and 1 responded well to touch on hand in a roughly somatotopic organization. The proportions of labeled neurons below the lesion in the ipsilateral cervical spinal cord (22.2% and 39.0%) were increased over neurons labeled in the short-term cases. Accordingly, we suggest that the second-order spinal cord pathway contributes to cortical reactivation, especially after incomplete DCLs and long recovery times.

Disclosures: **C. Liao:** None. **H. Qi:** None. **J.L. Reed:** None. **J.H. Kaas:** None.

Poster

709. Somatosensation: Plasticity

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Craig H. Neilsen Foundation fellowship to JLR, JHK

CIHR Foundation grant to MSS, MP

Title: Correlates of recovery after sensory loss with and without treatment in monkeys with dorsal column lesions

Authors: *J. L. REED[1], H.-X. QI[1], C.-C. LIAO[1], M. M. PAKULSKA[2], M. S. SHOICHET[2], J. H. KAAS[1];
[1]Psychology, Vanderbilt Univ., Nashville, TN; [2]Chem. Engin. and Applied Chem., Univ. of Toronto, Toronto, ON, Canada

Abstract: In a nonhuman primate model of spinal cord injury, unilateral lesions of the dorsal column (DC) disrupt sensory input from one hand to impair sensation and motor control. Task-specific rehabilitation (Rehab) to encourage hand use may promote functional recovery, and combining Rehab with appropriate interventions may further enhance recovery. Treatments incorporating the chondroitinase ABC (ChABC) enzyme to digest chondroitin sulfate proteoglycans in the extracellular matrix are studied for the potential to reduce barriers that prevent axons at the lesion site from growing to their targets. Rodent models yield promising results, and we collaborate here to investigate ChABC treatment using an affinity release hydrogel (Pakulska et al. 2015) in nonhuman primates to provide valuable information for clinical studies. We hypothesized that ChABC-hydrogel + Rehab would improve sensorimotor function sooner, and would result in more neurons with useful response properties in the cortex. Because the ChABC-hydrogel has been studied in rodents but not primates, we delivered the hydrogel alone to the cervical spinal cord in 1 adult male squirrel monkey (*Saimiri*) without inducing a DC lesion. Behavioral observations and histological processing of tissue for structural markers and microglia/macrophages indicated that the hydrogel was safe and did not produce undesirable side effects. We then obtained preliminary results from 2 adult male squirrel monkeys that received ChABC-hydrogel at the time of experimentally induced DC lesions. Promising data suggest that use of the impaired hand in the Rehab task occurred earlier with ChABC-hydrogel treatment than with Rehab only. After a 12-week Rehab period, we assessed neuron responses and topographic organization in both hemispheres of primary somatosensory cortex area 3b. Reactivation of the deprived area 3b was incomplete and retained abnormalities typically found after DC lesions. After single electrode mapping, we implanted microelectrode arrays into both hemispheres of 1 monkey and recorded responses to tactile stimulation. In our preliminary sample, responsive neurons had faster latencies and higher response rates after ChABC + Rehab compared to cases without treatment. These findings suggest that the ChABC treatment did not promote detrimental changes; however, and we found no evidence that ChABC-hydrogel healed the lesion within the 12 weeks studied. The spinal cord in both monkeys sustained extensive lesions of segment C5. By evaluating multiple measures, these

results represent initial studies to employ combination treatments with Rehab in a nonhuman primate model of spinal cord injury and sensory loss.

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Poster

709. Somatosensation: Plasticity

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Topic: D.03. Somatosensation: Touch

Support: Dept. Biotech., Govt. of India, #BT/PR7180/MED/30/907/2012 to NJ

CSIR-UGC: 19-06/2011(i)EU-IV to PH

NBRC Core Funds

Title: Reorganization of cuneate nucleus in macaque monkeys following chronic partial spinal cord injuries

Authors: *P. HALDER, N. JAIN;
Dept. of Systems Neurosci., Natl. Brain Res. Ctr., Gurgaon, India

Abstract: Cortical and sub-cortical somatosensory areas undergo large-scale reorganization following deafferentations. As a result of chronic unilateral dorsal column lesions, neurons in the deafferented hand region of cortical area 3b, secondary somatosensory area (SII), parietal ventral area (PV), and ventroposterolateral nucleus (VPL) of the thalamus respond to touch on the chin. However, extent and nature of reorganization of the brainstem dorsal column nuclei were not well understood. Here we determined the somatotopy of the cuneate nucleus, which normally receive afferent connections from the hand, in monkeys with chronic unilateral dorsal column injuries at cervical levels. We first mapped the somatosensory area 3b using multiunit mapping techniques. As expected, the chin representation showed an expansion into the hand area. Chin representation expanded medially up to a region where arm or occiput representations are found. Few hand-responsive neurons were found at some recording sites inside hand cortex due to minor sparing of dorsal column fibres. In the same monkeys, we mapped the dorsal column nuclei on the deprived side. Neurons showed responses to tactile stimulations to the chin throughout the cuneate nucleus. Few neurons in pars rotunda responded to touch on the hand as seen in area 3b. In addition, neck, shoulder, occiput and arm representations, which are normally confined to the dorsal and lateral parts of the cuneate nucleus, expanded into the ventral and

medial part of the nucleus. Normally inputs from digits and glabrous hand region are present in this region. Neurons at many recording sites had dual receptive fields either on chin and arm, or on chin and neck, shoulder or occiput. The present findings show that following dorsal column lesions, cuneate nucleus also undergoes large-scale reorganization, with expansion of chin representation, as well as of arm, neck, shoulder and occiput representation.

Disclosures: P. Halder: None. **N. Jain:** None.

Poster

709. Somatosensation: Plasticity

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Topic: D.03. Somatosensation: Touch

Support: NIH NEI R01EY022987-01

NIH NEI 2T32EY015387-11

NSF GRFP DGE-1148897

Title: Receptive fields and response properties of neurons in the S1 whisker representation of early blind short-tailed opossums

Authors: *D. L. RAMAMURTHY, L. A. KRUBITZER;
Ctr. for Neurosci., UC Davis, Davis, CA

Abstract: The mammalian neocortex has a remarkable capacity to alter its functional organization and connectivity in response to changing patterns of sensory input. In animals and humans deprived of visual input early in development, cortical areas normally associated with vision are recruited by spared sensory modalities. Further, there is also compensatory plasticity in the cortical areas associated with spared sensory modalities. Most previous studies examining cross-modal plasticity following blindness have examined plasticity within the auditory system. However, there have been a few reports of changes within the somatosensory cortex following blindness including the expansion of the S1 barrel fields in bilaterally enucleated rats. Previous studies in our laboratory have shown that there is an increase in the percentage of neocortex dedicated to S1 in bilaterally enucleated short-tailed opossums, and in some cases alterations in overall functional organization. The short-tailed opossum, *Monodelphis domestica*, provides a tractable animal model in which to investigate early cortical development because the immature state of the nervous system at birth allows for targeted manipulations to be performed *ex utero* very early in development. We used single-unit extracellular recording techniques to examine the

receptive fields and response properties of neurons in the S1 whisker representation of short-tailed opossums that were bilaterally enucleated at P4 (P0 in *Monodelphis domestica* is equivalent to E11 in the mouse and E12 in the rat). Preliminary data indicate that compared to sighted controls, S1 neurons in the whisker representation of these animals display lower stimulus-evoked firing rates and smaller receptive fields. These differences in S1 suggests that there is compensatory plasticity in primary somatosensory cortex of these early blind animals, which could either be a direct consequence of the early loss of visual input, or due to an differences in the extent to which early blind animals rely on whisker-mediated touch in the absence of vision.

Disclosures: D.L. Ramamurthy: None. **L.A. Krubitzer:** None.

Poster

709. Somatosensation: Plasticity

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Topic: D.03. Somatosensation: Touch

Support: MRC Grant G0901299

MR/N003896/1

Title: The role of CaMKII in spine dynamics and experience-dependent structural plasticity of L2/3 cortical neurons

Authors: *G. SEATON, A. M. DE HAAN, K. D. FOX;
Cardiff Univ., Cardiff, United Kingdom

Abstract: Functional plasticity studies to date in cortical layer 2/3 (L2/3) have demonstrated robust experience dependent potentiation (EDP) and long-term potentiation (LTP), whilst structural plasticity in these layers remains largely uncharacterized. Here we investigated the relationship between functional and structural plasticity in barrel cortex L2/3 neurons, by exploiting the CaMKII-T286A (T286A) mouse model, which lacks EDP and LTP, but retains LTD. Spines of T286A L2/3 basal dendrites were compared to those of wild-type animals (WT) for baseline turnover, formation and elimination in response to whisker deprivation, stability of newly formed spines and existing spine size fluctuations. In vivo 2-photon microscopy was used to image dendritic spines both before and after chessboard whisker deprivation. Baseline condition imaging (when animals are not whisker deprived) revealed T286A mutant mice have higher spine turnover rates compared to wild-type controls. However, T286A mutant mice

formed fewer new spines in response to whisker deprivation compared to WT mice. Furthermore, in T286A mice, new spines were less likely to persist beyond 8 days, which would suggest the probability of stable synapse formation may be reduced, in the absence of CaMKII autophosphorylation. (K. Svoboda 2006). Indeed T286A mice exhibit fewer "always persistent spines" (spines present throughout the entire imaging protocol), compared to WT controls. In comparison, following deprivation, WT control animals showed increased spine formation, destabilization of previously stable spines, and an increased number of new spines that persisted. Comparisons between pairs of dendrites at bifurcation points revealed that spines were specifically added to high formation branches in WT animals, whilst in T286A mutant mice, the few new spines that were formed in response to whisker deprivation showed no preference for dendrite branch. Increased baseline turnover and deficits in experience dependent new persistent spine formation in T286A animals coincided with a smaller range of spine size fluctuations compared to WT controls. In particular, large spines in T286 mice tended to be less extreme in size than in WT mice, which may be related to the T286 deficit in LTP. These results suggest CaMKII autophosphorylation plays a significant role in experience dependent dendritic spine restructuring in L2/3 neurones.

Disclosures: **G. Seaton:** None. **A.M. de Haan:** None. **K.D. Fox:** None.

Poster

709. Somatosensation: Plasticity

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 709.08/FF4

Topic: D.03. Somatosensation: Touch

Support: NIH 1R37 NS092367

Title: Environmental enrichment increases whisker responsiveness and alters somatotopy in L/3 of somatosensory cortex

Authors: ***A. M. LEMESSURIER**, S. CHEN, K. M. MCCLAIN, D. E. FELDMAN;
Helen Wills Neurosci. Inst., UC Berkeley, Berkeley, CA

Abstract: The whisker map in L2/3 of mouse primary somatosensory cortex has very high local scatter, such that neighboring neurons, intermixed within a column, are often tuned to different nearby whiskers (Sato & Svoboda 2010; Clancy et al., 2015). This "salt and pepper" organization was discovered in mice housed in standard laboratory conditions, which may represent a deprived environment. Here we test how sensory experience regulates this micro-organization within L2/3. We raised C57BL/6 mice with environmental enrichment (EN),

including tactile objects and 2-3 littermates, or in normal housing (NH), with 1 littermate but no other objects. Objects were introduced at weaning (P21) and were exchanged regularly to encourage whisker exploration. At age P50 ± 10 days, we measured whisker responses and 9-whisker tuning curves (somatotopic tuning) in L2/3 using 2-photon imaging of virally expressed GCaMP6s through a chronic cranial window. We observed robust changes in whisker-responsiveness and local somatotopy with enrichment. In EN mice, spontaneous activity was increased over NH mice, and cells showed stronger dF/F responses to their best whisker. In NH mice, neurons were not spatially clustered by whisker tuning—neurons tuned to the same whisker were not closer together than random pairs of neurons in the imaging field, which was roughly the area of one column. However, in EN mice, co-tuned neurons were significantly spatially clustered relative to randomly chosen neurons ($p<0.01$). This suggests either more accurate columnar somatotopy, or more homogeneous tuning neighborhoods within the column. To measure these effects specifically in pyramidal neurons, we repeated the experiments in 45 ± 5 day old Drd3-Cre mice, which expressed GCaMP6s in a subset of L2/3 pyramidal neurons. In NH Drd3-Cre mice, only 6.6%± 2.7% of GCaMP6-expressing pyramidal neurons were responsive to any whisker, whereas 51%± 13% were responsive in EN littermates. In enriched Drd3-Cre mice, co-tuned pairs of cells were more spatially clustered than random pairs ($p<0.01$), similar to the C57BL/6 cohort. These findings demonstrate a strong impact of sensory experience on the spatial organization of the L2/3 whisker map.

Clancy KB, Schnepel P, Rao AT, Feldman DE. Structure of a single whisker representation in layer 2 of mouse somatosensory cortex. J Neurosci. 2015;35(9):3946-58.
Sato TR, Svoboda K. The functional properties of barrel cortex neurons projecting to the primary motor cortex. J Neurosci. 2010;30(12):4256-60

Disclosures: **A.M. Lemessurier:** None. **S. Chen:** None. **K.M. Mcclain:** None. **D.E. Feldman:** None.

Poster

709. Somatosensation: Plasticity

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 709.09/FF5

Topic: D.03. Somatosensation: Touch

Support: COBRE Stroke Recovery Research Center

Title: Toward a murine model to image the remapping of synaptic inputs after stroke

Authors: *M. LEVY, R. I. GRANT, C. BURTON, A. Y. SHIH;
neurosciences, MUSC, Charleston, SC

Abstract: After a stroke in the somatosensory cortex, the function of tissues lost to injury will remap into regions surrounding the infarct. This remapping forms the basis for functional recovery after stroke, but its mechanism remains poorly understood. Anatomical studies have shown that dendritic spines in layer 1 become more labile during the period of remapping, suggesting that the cortex is a locus of plasticity (Brown et al. J Neurosci 2007). However whether these anatomical changes correspond to new sensory inputs has not been demonstrated. Following recent advances in subcellular calcium imaging (Chen et al. Nature 2013; Varga et al. PNAS 2012), we developed a murine model where post-stroke remapping can be imaged longitudinally *in vivo* at the level of individual spines. Neurons in all layers of the barrel cortex except layer 4 were sparsely labeled with a genetically-encoded calcium sensor (GCaMP6s) using adeno-associated viruses. Spine calcium responses to stimulations of the barrel's principal whisker or of surrounding whiskers were imaged over multiple days using two-photon microscopy. In each imaging session (typically lasting 2h), we could sample the activity of ~100 spines on a single neuron. We focused on pyramidal neurons in layer 2 and 5 which were brightly labeled. At the spine level, spontaneous as well as sensory-evoked calcium transients were sparse. Approximately 1/10 spines displayed reliable responses to whisker stimulation. In agreement with previous results, most responsive spines preferred stimulation of the barrel's principal whisker, while the remainder responded to both whiskers or to the surrounding whisker alone. We are currently studying the stability of spine whisker preference over the course of several weeks. We will next examine the plasticity of synaptic inputs in peri-infarct tissue after a barrel is deleted by photothrombotic stroke.

Disclosures: **M. Levy:** None. **R.I. Grant:** None. **C. Burton:** None. **A.Y. Shih:** None.

Poster

709. Somatosensation: Plasticity

Location: Halls B-H

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Program#/Poster#: 709.10/FF6

Topic: D.03. Somatosensation: Touch

Support: SNSF Grant CRSII3_154453

SNSF Grant 31003A_153448

SNSF Grant 51AU40_125759

IRP and Hans Wilsdorf Foundation

Title: LTP in the mouse barrel cortex driven by cooperative lemniscal and paralemniscal pathway activity

Authors: *L. E. WILLIAMS, A. HOLTMAAT;
Fundamental Neurosci. and Ctr. for Neurosci., Univ. of Geneva, Geneva, Switzerland

Abstract: Long term potentiation (LTP) is thought to underlie changes in synaptic efficacy as observed during cortical synaptic network plasticity and learning. Our lab has previously demonstrated *in vivo* that repeated sensory input, in the form of 8-Hz rhythmic whisker stimulation (RWS), induces synaptic LTP in layer (L) 2/3 pyramidal cells of the somatosensory barrel cortex (BC) [Gambino et al., Nature, 2014. 10.1038/nature13664]. Whisker sensory information is primarily processed by the lemniscal pathway, which comprises neurons in the ventral posteromedial (VPM) thalamus that mainly project to BC L4 and L5B. L4 cells in turn synapse on L2/3 pyramidal cells. Whisker stimuli also recruit activity in paralemniscal circuits, which contain projections from the posteromedial complex of the thalamus (POm) to BC L1 and L5A. Our lab has found that RWS evokes prolonged coactivity of these two pathways, which is necessary to drive sensory-evoked LTP in the absence of somatic spikes. However, the exact nature of the synaptic circuits remains elusive. Here, using whole-cell patch clamp recordings in thalamocortical slices we tested if repeated coincident activity of L4 and POm synapses onto L2/3 pyramidal cells is sufficient to drive LTP. Activity of L4 and POm synapses was paired by electrical stimulation of L4 and optical stimulation of channelrhodopsin-2 (ChR2) expressing POm axons. Rhythmic (8Hz) coincident pairing of activity in these two circuits efficiently induced LTP of postsynaptic potentials (PSPs) in the L4-to-L2/3 synapses. Paired stimulation while suppressing POm neurotransmission using Designer Receptors Exclusively Activated by Designer Drugs (DREADDS) pharmacogenetics, or while blocking NMDA receptors, failed to induce LTP. We are currently testing to what extent GABAergic inhibition on L2/3 dendrites regulates this form of LTP. L2/3 pyramidal dendrites are efficiently inhibited by somatostatin (SST) expressing interneurons, which in turn are inhibited by vasoactive intestinal peptide (VIP) expressing interneurons. Thus, VIP cells are thought to disinhibit L2/3 pyramidal dendrites, which could gate activity- dependent synaptic plasticity. We find that optical stimulation of ChR2-expressing POm axons evokes PSPs in VIP neurons. Preliminary data suggest that inhibition of VIP or SST interneuron activity using DREADDS in VIP-CRE and SST-CRE mouse transgenic lines bidirectionally alters LTP. Our data identifies excitatory and disinhibitory microcircuits whose synergistic activity may facilitate sensory-driven LTP in the barrel cortex.

Disclosures: L.E. Williams: None. **A. Holtmaat:** None.

Poster

709. Somatosensation: Plasticity

Location: Halls B-H

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Program#/Poster#: 709.11/FF7

Topic: D.03. Somatosensation: Touch

Support: NIH Grant NS089683

Title: Brain-derived neurotrophic factor and caspase activity in mouse barrel cortex following whisker plucking

Authors: *S. A. MARIK, C. D. GILBERT;
Rockefeller Univ., New York, NY

Abstract: Brain-derived neurotrophic factor (BDNF) plays a key role in development and plasticity of the central nervous system. We previously reported that death receptor 6 (DR6) and amyloid precursor protein (APP) are required for axonal pruning in adult somatosensory cortex following manipulation of sensory experience (e.g. whisker plucking). While neurotrophin withdrawal leads to activation of the DR6/APP pathway in the developing brain, the link between neurotrophins and the DR6/APP pathway in adult plasticity has not been determined. Here we confirm previous reports that an elevation in BDNF within the lesion projection zone (LPZ) occurs following sensory deprivation. Furthermore, we directly tested if artificially increasing BDNF levels would result in the expression of key members of the DR6/APP pathway by injecting a CMV.GFP.2A.GFP AAV into mouse somatosensory cortex. Immunohistochemical labeling of components of the apoptotic pathway showed upregulation of proBDNF, DR6 and APP in neurons surrounding BDNF expressing cells. APP was upregulated mainly in layer 2. As expected, proBDNF expression occurred in BDNF infected neurons; however, unexpectedly neurons neighboring GFP positive neurons also expressed proBDNF. Caspase 3 expression was observed within in a small population of BDNF expressing neurons throughout the injection. We examined expression of cleaved caspase 3 in WT and CREmKI mice, which contain a mutation in promoter IV of the BDNF gene. Two days following whisker plucking cleaved caspase 3 is detectable within the barrel cortex of WT mice but not detected in CREmKI mice. Taken together these results suggest a role for BDNF in the DR6/APP pathway and the resultant experience dependent axonal pruning in the adult.

Disclosures: S.A. Marik: None. **C.D. Gilbert:** None.

Poster

709. Somatosensation: Plasticity

Location: Halls B-H

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Program#/Poster#: 709.12/FF8

Topic: D.03. Somatosensation: Touch

Support: MOST 104-2811-B-182-031

MOST 104-2314-B-182-050

CMRPG5C0073

Title: Neuroplasticity in the primary sensory cortical neurons following facial nerve reconstruction in adult rats

Authors: *J.-J. HUANG[1,4,2], J.-L. CHEN[4,1], C.-H. LIN[3,2], Y.-C. PEI[1,4,2];
[1]Dept. of Physical Med. and Rehabil., [2]Ctr. for Vascularized Composite Allotransplantation, [3]Dept. of Plastic and Reconstructive Surgery, Chang Gung Mem. Hosp., Taoyuan, Taiwan; [4]Sch. of Med., Chang Gung Univ., Taoyuan, Taiwan

Abstract: Sensory-motor recovery is a spontaneous phenomenon that occurs after peripheral nerve injuries, such as cutting, blunt, laceration injury and nerve transplantation, and is mediated by neuroplasticity. Even though the return of neuronal activities in peripheral nerves and trigeminal ganglia have been observed, the neuronal mechanisms mediated by the thalamus and cerebral cortex remain unknown. To this end, we used whisker-to-barrel model in rodents to evaluate the alteration of neuronal tuning following a neurotomy and neurorrhaphy surgery, an approach that is analogous to a nerve reconstruction surgery in patients. We used a piezo-actuator-based whisker stimulator to bend the whisker at a variety of directions and speeds. We applied extra-cellular recording using micro-wire electrode arrays to record neuronal spiking activities in the barrel cortex in response to whisker stimulation in adult rats. The recording was performed before and after the neurotomy and neurorrhaphy surgery with a follow-up period up to 4 to 6 weeks post-surgery. The results showed that all units lost their sensory responses immediately after the surgery and, surprisingly, the sensory responses could recover as early as 7 days post-surgery. Further follow-up showed that one third of the units regained their whisker tuning that is analogous to that before the surgery. The onset latency of neuronal responses to whisker stimulations prolonged in the early recovery period and gradually decreased in the late recovery period. In conclusion, the present study reveals the neuronal mechanisms that account for the functional recovery following nerve reconstruction in the whisker-to-barrel model.

Disclosures: **J. Huang:** None. **J. Chen:** None. **C. Lin:** None. **Y. Pei:** None.

Poster

709. Somatosensation: Plasticity

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 709.13/FF9

Topic: D.03. Somatosensation: Touch

Support: KAKENHI(26430022)

KAKENHI(25115721)

Title: Physiological and morphological roles of endocannabinoid 2-arachidonoylglycerol in the developing neuronal circuit in the mouse barrel cortex

Authors: *C. ITAMI[1], K. SAKIMURA[2], M. KANO[3], F. KIMURA[4];
[1]Saitama Med. Univ., Saitama, Japan; [2]Dept of Cell Neurobiol, Brain Res. Inst, Niigata Univ., Niigata, Japan; [3]Dept of Neurophysiol, Grad Sch. of Med, Univ. of Tokyo, Tokyo, Japan; [4]Dept of Mol Neurosci, Grad Sch. of Med, Osaka Univ., Osaka, Japan

Abstract: Recent studies indicated that endocannabinoids play important roles in the development of neuronal circuits both physiologically and morphologically. In the rodent barrel cortex, we have shown that type 1 cannabinoid receptors (CB1R) cause the long-term depression (LTD) component of spike timing-dependent plasticity (STDP) at the thalamocortical as well as L4-L2/3 synapses. We also demonstrated that CB1R knockout (CB1R-KO) mice show disorganized thalamocortical projections. Thus, endogenous CB1R signalling is important in translating physiological changes into morphological consequences. Anandamide and 2-arachidonoylglycerol (2-AG) are regarded as two major endocannabinoids, but to what extent each substance is involved in the cerebral cortex is still obscure. We show here that observed physiological and morphological effects are accounted for by 2-AG as the major endocannabinoid, using mutant mice lacking a 2-AG synthesizing enzyme, diacylglycerol lipase α (DGLα). We found that timing-dependent LTD (tLTD) was completely absent at L4-L2/3 as well as thalamocortical synapses in DGLα KO mice. Rather, KO animals and heterozygotes showed slight potentiation, possibly revealing occluded tLTP. In addition, we observed morphological abnormality in the thalamocortical projections stained by DiI at P12 in DGLα KO mice. Individual axons did not terminate within barrels as in wild type mice; instead, there were significant invasions of thalamocortical afferents into L2/3 as in CB1R KO mice. From theses results, we conclude that 2-AG works as a major endogenous ligand to produce physiological and morphological effects acting on CB1R in the mouce barrel cortex.

Disclosures: **C. Itami:** None. **K. Sakimura:** None. **M. Kano:** None. **F. Kimura:** None.

Poster

709. Somatosensation: Plasticity

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 709.14/FF10

Topic: D.03. Somatosensation: Touch

Support: Research development funds to JK

Title: Effectiveness of a robot-aided proprioceptive training program with vibro-tactile feedback on proprioception in chronic stroke participants

Authors: *I.-L. YEH[1], N. ELANGOVAN[1], J. HOLST-WOLF[1], A. CUPPONE[3], J. KONCZAK[1,2];
[1]Human Sensorimotor Control Lab, Sch. of Kinesiology, [2]Program of Neurosci., Univ. of Minnesota, Minneapolis, MN; [3]Dept. of Robotics, Brain and Cognitive Sciences,, Inst. Italiano di Tecnologia, Genoa, Italy

Abstract: Proprioception refers to the perception of limb motion or position and the orientation of one's body in space. Nearly 50% of stroke survivors showed proprioceptive deficits (e.g. Carey & Matyas 2011), which is associated with poor upper limb motor function impairing many activities of daily living. Thus, improving proprioception could be an additional route to enhance motor recovery. We designed a robot-aided training regimen that required users to make active wrist movements without vision. User grasped the handle of the robot and performed wrist adduction/abduction movements to tilt a virtual board on which a ball rolled. Aim was to roll the ball to a target on the board. Real-time, vibro-tactile feedback about joint position and velocity was provided to the forearm. METHODS: Two functionally independent yet with limited affected arm use, chronic stroke participants were recruited (Fugl-Meyer Assessment: 65/66 & 27/66). Four healthy participants (mean age ± SD: 26.4 ± 3.3 years) served as controls. Participants completed two training sessions on two consecutive days (total training time: 1 hour). A familiarization phase with vision was completed prior to training, while training was performed without vision. Task difficulty increased as the participants succeeded. Assessment was conducted before, immediately after, and two days after the intervention. Outcome measures were 1) wrist position sense acuity defined as the just-noticeable-difference threshold of wrist abduction/adduction and 2) the latency (time to peak) of a somatosensory-evoked potential proprioception-related component (N30). Controls were assessed only before and after the intervention. RESULTS: The more severely impaired stroke participant improved and maintained his wrist position acuity (Pretest: 5.4°; Day 2 (End of training): 3.3°; Day 5: 2.3°). The less affected stroke participant did not show proprioceptive deficits but performed within the range of the control group (Pretest: 0.8°; Day 2: 0.8°, Day 5: 1.2°; Control group: Pretest mean ± SD: 2.0° ± 1.2°; Day 2 mean ± SD: 1.4° ± 0.5°). The N30 latencies were within the known

normal range throughout the tests in both groups. CONCLUSION: Robot-aided proprioceptive training may improve proprioceptive acuity in chronic stroke participants with known proprioceptive impairment. If the effectiveness on improving the proprioceptive and motor function is substantiated in a larger population, the proposed training could be a treatment approach for clinical practice. REFERENCES: Carey LM & Matyas TA. Frequency of discriminative sensory loss in the hand after stroke in a rehabilitation setting. J Rehabil Med. 2011;43(3):257-63.

Disclosures: I. Yeh: None. **N. Elangovan:** None. **J. Holst-Wolf:** None. **A. Cuppone:** None. **J. Konczak:** None.

Poster

709. Somatosensation: Plasticity

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 709.15/FF11

Topic: D.03. Somatosensation: Touch

Support: Gottfried Wilhelm Leibniz Price

Deutsches Zentrum für Neurodegenerative Erkrankungen

Bernstein Center for Computational Neuroscience Berlin

Humboldt Universität zu Berlin

Title: A comparative study of genital representation in the primary somatosensory cortex of mammals

Authors: *S. LAUER[1], C. LENSCHOW[2], M. BRECHT[2];
[1]Bernstein Ctr. For Computat. Neurosci., Berlin, Germany; [2]Bernstein Ctr. for Computat. Neurosci., Berlin, Germany

Abstract: Mammals show sexually dimorphic external genitalia. Little is known about the representation of these in the primary somatosensory cortex (S1). Recent anatomical and physiological studies in the rat have identified a region in S1, which responded to genital stimulation (Lenschow et al., 2016). In rats genital cortex was sexually monomorphic and showed expansion during puberty. In the current study, we addressed the following questions: (i) Is the distinctive representation of penis/clitoris conserved across species? (ii) Is genital representation sexually monomorphic across species? (iii) Can growth of genital cortex also be observed in other species? (iv) Is there a correlation between socio-sexual behavior and genital

representation? To answer these questions, we performed cyotchrome oxidase staining of the flattened cortex and identified a distinctive genital representation in eight further species (Mouse, Mongolian Gerbil, Syrian Hamster, Degu, Guinea Pig, American Chipmunk, Chinchilla, Rabbit). Genital cortices were sexually monomorphic. Four species (Mouse, Hamster, Rabbit, Gerbil) were investigated for puberty related expansion of the genital cortex and we observed a 1.4 fold to 3.1 fold increase, similar to that reported in rats (Lenschow et al., 2016). The relative size of genital cortex varied between the species (0.41 to 1.73% of the area of somatosensory cortex), with monogamous animals typically exhibiting a smaller genital cortex compared to polygamous animals. Comparisons between solitary and social living species did not reveal any differences. Remarkably, relative testicle size (a robust evolutionary indicator of sperm competition) and relative size of genital cortex area was highly correlated. Our results identify conserved features of genital cortex (position, sexual monomorphism, pubertal expansion, erect posture of the penis). Species differences point to an important role of sexual selection in the evolution of genital cortex - to our knowledge this is the first time that effects of sexual selection on a cortical region have been documented.

Disclosures: **S. Lauer:** None. **C. Lenschow:** None. **M. Brecht:** None.

Poster

710. Auditory System: Periphery

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 710.01/FF12

Topic: D.05. Audition

Support: the Ministry of Education, Culture, Sports, Science, and Technology (Japan)

Title: Intrinsic harmonics in the human cochlea revealed by 7 Tesla MRI

Authors: K. SUZUKI[1], M. WATANABE[1], H. MATSUZAWA[1], K. NAKADA[1], I. L. KWEE[2], *T. NAKADA[1,2];
[1]Brain Res. Inst, Univ. of Niigata, Niigata, Japan; [2]Univ. of California, Davis, Davis, CA

Abstract: The classical model of the cochlea views it as a “spectral analyzer” made of a “dispersive transmission line” where different frequency components travel at different speeds (travelling wave). The basilar membrane (BM) is topographically organized so that the BM at the specific location resonates with its characteristic frequency. Although this linear concept and model has provided a powerful approximation, modern investigations revealed that the behavior of the cochlea is highly non-linear on which its functionality appears to be strongly dependent. Since, in principle, activities of the inner hair cell (IHC) and, hence, spiral ganglionic acoustic

neuron linearly correlate with the BM displacement, the non-linear behavior of the BM with respect to auditory input primarily is believed to arise from active outer hair cell (OHC) modification on passive responses of endolymph towards input signals. Currently, there are two leading hypotheses related OHC function, referred to as the non-linear resonant tectorial membrane (NL-RTM) and the cochlear amplifier (CA) model. Unfortunately, they still remain far from providing a perfect description of cochlear functionality; especially when one considers the significance of formants in human language processing. Using functional magnetic resonance imaging (fMRI), we have previously demonstrated that behavior of the human primary motor cortex can be effectively modeled by ensemble behavior of limit cycle oscillators and entrainment (Entropy 17: 7596-7607, 2015). Should OHC possess properties of a self-excited van der Pol (limit-cycle) oscillator, it could help the BM to synchronize with subharmonic driving forces passively brought about by auditory stimuli by the entrainment mechanism. To test this hypothesis, we performed *in vivo* analysis of the BM response in the human cochlea using 7 Tesla MRI. Endolymphatic fluid motion concomitant with BM displacements was detected as signal alteration of steady-state free precession imaging technique. The results unequivocally exhibited that stimulus sounds (pure tone and narrow-band chirp, centered at 3 kHz or 4 kHz) introduced the BM response not only at the location corresponding to the characteristic frequency but also those for its overtones. The study strongly indicated that human cochlea has an intrinsic sensitivity to pure tone harmonics, which is likely driven by BM resonance with subharmonics (1/2, 1/3, 1/4, ⋯) of its characteristic frequency inherent to non-linear dynamics.

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Poster

710. Auditory System: Periphery

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Program#/Poster#: 710.02/FF13

Topic: D.05. Audition

Support: ICMR grant 45/19/2012-ANA/BMS

AIIMS Grant 218/A-218/2013/RS

Title: Spiral ganglion in an aged adult human cochlea

Authors: C. KAUR[1], T. G. JACOB[1], T. C. NAG[1], A. THAKAR[1], D. BHARDWAJ[1], *T. ROY[2]; [1]Dept. of Anat., All India Inst. of Med. Sci. New Delhi, New Delhi, India; [2]All India Inst. Med. Sci., New Delhi, India

Abstract: Constant exposure to occupational and environmental noise impairs the hearing ability and degree of impairment and its prevalence increases with age. One major cause of age related hearing loss is the loss of Spiral Ganglion Neurons (SGNs) that convey nerve impulses from the hair cells of the cochlea to the cochlear nucleus for audition. Two important neurotransmitters that are involved in the auditory pathway are the inhibitory GABA and the excitatory Glutamate (one of its common receptors being NMDAR-2B in SGN). Earlier, we had estimated that the young adult (between 21-30 years) have on an average 27,485 ± 3251.3 neurons, and that there were an equivalent number of GABA and NMDAR-2B positive neurons in the same cases.In this study, we estimated the total number of SGNs in the cochlea of an elderly adult and also studied the expression of GABA and NMDAR-2B in the same case. Temporal bone specimen of a 70 year old individual was obtained from the mortuary at All India Institute of Medical Sciences, New Delhi, with approval from the Institute's Ethics committee. It was fixed in buffered formalin, dissected, decalcified, cryoprotected and serially sectioned (30µm) in the coronal plane. Every 7th section was stained by cresyl violet, immunostained with GABA (Abcam, ab86186; 1:1000), NMDAR 2B (Abcam, ab93610; 1:1000) using standard protocol. The total number of SGNs (Optical Fractionator), SG volume (Cavalieri) and the volume of soma and its nucleus (Nucleator) were estimated with the StereoInvestigator software (Microbrightfield Inc. VT, USA).The estimated total number of neurons was 14730, total volume of the SG was 1.32 mm^3, and the volume of the soma and its nucleus were 2913µm^3 and 144µm^3 respectively. The expression of GABA and NMDAR 2B was reduced when compared to its expression in younger adults. Hence, we show that in the elderly, there is a significant reduction in the number of SGNs and a decreased expression of GABA and NMDAR-2B in the SGNs. These findings need to be confirmed in a larger population, but these preliminary results suggest that aging probably causes a reduction in the number of SGNs and also the efficacy of the surviving cells, since their functioning is dependent on the availability of the neurotransmitters GABA and glutamate.

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Poster

710. Auditory System: Periphery

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 710.03/FF14

Topic: D.05. Audition

Title: Significantly improved recovery of severe noise-induced hearing loss by the orally available, clinical drug candidate SENS-401

Authors: *J. DYHRFJELD-JOHNSEN, M. PETREMANN, L. BEYNAC, A. BROUSSY; Sensorion SA, Montpellier, France

Abstract: Sensorineural hearing loss through acoustic damage to the sensory hair cells, synapses and neurons of the cochlea is among the most common types of permanent hearing loss for adults (American Speech-Language-Hearing Association - ASHA; Hearing Loss Association of America - HLAA). Currently no approved pharmaceutical treatment exists for sudden hearing loss and recent meta-analysis of the standard-of-care, off-label use of corticosteroid therapy have concluded that neither systemic nor intratympanic administration has any significant treatment effect (Crane et al. 2015, Laryngoscope 125(1):209-17).

SENS-401 is small molecule, clinical drug candidate which can be administered orally and achieves significant local exposure (50-250 nM) in perilymph and inner ear tissue lasting at least 6 hours after a single oral administration in naïve rats.

Following baseline audiometry (ABRs at 8/16/24 kHz; DPOAEs at 4/8/16/24/32 kHz), 7 week old awake and behaving male Wistar rats were exposed to 120 dB octave band noise (8-16 kHz) for 2 hours on a slowly rotating platform in a sound-attenuating cubicle. SENS-401 at doses of 5 mg/kg, 10 mg/kg and 20 mg/kg or placebo treatment was initiated after the end of acoustic trauma exposure using oral gavage continued daily for 14 days.

In animals suffering initial severe hearing loss (ABR threshold shifts > 55 dB or otoacoustic emissions detectable only at 2 or fewer test frequencies at t=24h), daily SENS-410 treatment dose-dependently reduced permanent ABR threshold shifts from baseline to D14 from 52.1-59.3 dB in the placebo group down to 33.3-49.2 dB in the 2 highest dose SENS-401 treated groups. This corresponds to an improvement from “moderately severe” to “mild/moderate” hearing loss according to ASLHA criteria. ABR threshold recovery from 24h to D14 was also dose-dependently improved from 7.1-12.9 dB in the placebo group to up to 20.8-29.2 dB in the 2 highest dose SENS-401 treated groups. DPOAE amplitude recovery from 24h to D14 was similarly dose-dependently improved, with placebo treated animals particularly showing near complete and permanent loss of otoacoustic emissions at the most sensitive, higher tested frequencies (16/24/32 kHz), while SENS-401 treated animals showed partial recovery in the 2 highest dose groups.

These results demonstrate that daily, oral administration of the small molecule clinical candidate drug SENS-401 leads to significant local drug exposure and strongly enhances recovery in rats suffering from severe, acute noise-induced hearing loss.

Disclosures: J. Dyhrfjeld-Johnsen: None. **M. Petremann:** None. **L. Beynac:** None. **A. Broussy:** None.

Poster

710. Auditory System: Periphery

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 710.04/FF15

Topic: D.05. Audition

Support: P30 ES020957

WSU Faculty start-up

Title: LMO4 downregulation is a critical factor in cisplatin-mediated ototoxicity

Authors: *S. JAMESDANIEL[1], R. RATHINAM[1], W. NEUMANN[2];
[1]Inst. of Envrn. Hlth. Sci., Wayne State Univ., Detroit, MI; [2]Dept. of Pharmaceut. Sci., Southern Illinois Univ. Edwardsville, Edwardsville, IL

Abstract: Ototoxicity is a major side-effect of cisplatin, a highly effective anti-cancer drug. Several studies have indicated that oxidative stress plays a causal role in mediating cisplatin-induced ototoxicity. We reported that cisplatin treatment leads to nitration and downregulation of a transcription regulator, Lim domain only 4 (LMO4), in the cochlea. To evaluate the critical role of LMO4 in cisplatin ototoxicity we genetically manipulated the expression levels of LMO4 in UBOC1 cells, which are immortalized auditory sensory epithelial cells derived from C57BL/6 mice. Transient overexpression of LMO4 using the mammalian expression vector pRK5, significantly attenuated cisplatin-induced decrease in cell viability ($p<0.0001$), as indicated by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. CRISPR/Cas9 knock-out of LMO4 significantly worsened cisplatin-induced cytotoxicity ($p<0.01$), as indicated by the cell counts using trypan blue stain. Furthermore, auditory brainstem responses of CBA/J mice indicated that co-treatment with a peroxynitrite decomposition catalyst (SRI110), which attenuated cisplatin-induced decrease in LMO4 and apoptosis in UBOC1 cells, mitigated the cisplatin-induced shift in hearing thresholds ($p<0.01$, at 8 kHz). These data suggest that LMO4 probably has a protective role in the organ of Corti at normal or higher levels. However, cisplatin-induced decrease in LMO4 protein levels compromises the anti-apoptotic cellular defenses thereby facilitating cochlear apoptosis. We conclude that cisplatin-induced nitration and downregulation of LMO4 plays a critical role in facilitating the ototoxic effects. This study was supported by WSU faculty start-up funds and NIEHS P30 Grant (P30 ES020957).

Disclosures: **S. Jamesdaniel:** None. **R. Rathinam:** None. **W. Neumann:** None.

Poster

710. Auditory System: Periphery

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Topic: D.05. Audition

Support: DoD Award W81XWH-12-1-0492

NIH institutional training grant NIHT32-DC00011

Title: FGF-dependent otic induction in inner ear organoid derivation from $Pax2^{EGFP/+}$ mouse embryonic stem cells

Authors: *S. A. SCHAEFER, R. K. DUNCAN;
Univ. of Michigan, Ann Arbor, MI

Abstract: Stem cells represent a potential source of cell types that are difficult to obtain for study in the lab. The sensory hair cells of the inner ear present a particular challenge: they are relatively scarce and concealed by the temporal bone. Therefore, a major goal of hearing research is the efficient production of hair cells for downstream applications such as replacement therapy, developmental studies, and drug discovery. Recently, a method for producing inner ear organoids from aggregates of mouse embryonic stem (ES) cells was established. This method recapitulates basic signaling events of otic development (Koehler et al., 2013). A key event is otic induction, when progenitors upregulate Pax2 in response to fibroblast growth factors (FGFs) and commit to otic fate. This event is mediated by the ERK pathway downstream of FGF receptors in chickens and zebrafish (Wang et al., 2015; Yang et al., 2013). However, this has not been shown in mammals, and FGFs are known to signal through additional pathways including AKT and PLCγ. Understanding the mechanism of otic induction would permit more efficient recapitulation of mammalian inner ear development. To study otic induction in a mammalian model, we adopted the inner ear organoid method. We used $Pax2^{EGFP/+}$ mouse ES cells with EGFP inserted upstream of one Pax2 allele. This provided a report of Pax2 upregulation during FGF-driven otic induction. FGF2 was applied at 0, 5, 25, and 100 ng/mL, and ERK phosphorylation was assayed in some aggregates via Western blotting. Others were observed over several days as they developed internal $EGFP^{+}$ otic vesicle-like structures, which pushed outward and expanded into large protrusions. $EGFP^{+}$ cells became concentrated at the organoid regions (i.e., the protrusion-aggregate borders). Organoids were fixed with 4% PFA and stained by immunofluorescence to confirm presence of hair cell-like cells at day 20. Finally, the effect of Pax2 dose on organoid formation was examined using $Pax2^{EGFP/EGFP}$ mouse ES cells, which express a lower amount of Pax2. The degree of ERK phosphorylation correlated positively with FGF2 dose within 3 hours of application. In addition, the formation of organoids correlated positively with both FGF2 and Pax2 dose. Organoids contained $Myo7a^{+}$ cells with F-actin^{+}

stereocilia-like structures. These cells were able to rapidly uptake the styryl dye FM4-64, indicating functional mechanotransduction channels. These results suggest that FGF2 drives Pax2 expression and otic induction through the ERK pathway in a mammalian system. Inhibition of other FGF-driven pathways may promote otic induction, facilitating higher yields of hair cells.

Disclosures: **S.A. Schaefer:** None. **R.K. Duncan:** None.

Poster

710. Auditory System: Periphery

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Topic: D.05. Audition

Support: AMED-CREST, AMED

Title: Generation of a deaf model mouse by application of the optogenetic approach to the stria vascularis of the inner ear

Authors: ***M. P. SATO**[1,2], H. TAIGA[1], H. NIN[1,3], G. OGATA[1,3], T. YOSHIDA[1,3,6], K. HORI[1], S. KOMUNE[7], S. UETSUKA[1,8], C. SAMUEL[4,9], M. MASUDA[10], T. WATABE[11], S. KANZAKI[11], K. OGAWA[11], H. INOHARA[8], S. SAKAMOTO[5], H. TAKEBAYASHI[12], K. F. TANAKA[13], H. HIBINO[1,3,9];
[1]Niigata Univ. Sch. of Med., Niigata City / Niigata, Japan; [2]Otolaryngology, Kinki Univ. Sch. of Med., Osaka, Japan; [3]Ctr. for Transdisciplinary Res., [4]Electrical and Electronics Engin., [5]Mechanical and Production Engin., Niigata Univ., Niigata City / Niigata, Japan; [6]Otorhinolaryngology, Grad. Sch. of Med. Sciences, Kyushu University,, Fukuoka, Japan; [7]Otolaryngology-Head and Neck Surgery, Yuaikai Oda Hosp., Saga, Japan; [8]Otorhinolaryngology–Head and Neck Surgery, Grad. Sch. of Medicine, Osaka Univ., Osaka, Japan; [9]AMED-CREST, Niigata City / Niigata, Japan; [10]Otolaryngology, Kyorin Univ. Sch. of Med., Tokyo, Japan; [11]Otolaryngology, Head and Neck Surgery, Keio Univ. Sch. of Med., Tokyo, Japan; [12]Neurobio. and Anat., Grad. Sch. of Med. and Dent. Sci., Niigata City / Niigata, Japan; [13]Neuropsychiatry, Sch. of Medicine, Keio Univ., Tokyo, Japan

Abstract: Sensorineural hearing loss (SNHL) stems primarily from by disorder of the cochlea of the inner ear, and this disease provides a social impact due to lack of effective medical therapies. In the cochlea, mechanical energy of sounds is converted to electrical signals by sensory hair cells and thereafter the information is transmitted to the brain through the auditory nervous system. The mechanoelectrical transduction is greatly enhanced by an endocochlear potential (EP) of +80 mV in a K^+-rich extracellular fluid, endolymph. Hair cells expose their

mechanoelectrical apparatus to the endolymph. The positive potential of the endolymph depends on membrane potentials of the stria vascularis, an epithelial-like tissue in the cochlear lateral wall. Disorders of the stria are suggested to be involved in various idiopathic SNHL such as sudden deafness and Ménière's disease. These diseases are characterized by sudden onset and/or short-term hearing fluctuation. Such phenotypes in human have not been reproduced in animals by pharmacological methods and conventional gene-targeting techniques. Here we have applied optogenetical approach to the stria vascularis. We analyzed the mice that express channelrhodopsin-2 (ChR2), a blue light-gated nonselective cation channel, under the control of proteolipid protein promoter. A blue-light illumination to the cochlea of ChR2-expressing mice resulted in moderate elevation of auditory brainstem response (ABR) thresholds throughout the audible frequency range by ~20 dB in 3 min. After cessation of the illumination, the hearing recovered to the initial level in 5 min. Immunolabeling assays revealed that ChR2 occurred in intermediate cells (ICs) of the stria as well as glial cells in the nervous system. Whole-cell patch-clamp experiments showed that the illumination rapidly depolarized that membrane potential of IMs by approximately 20 mV. In *in vivo* electrophysiological recordings, the EP was reduced by roughly 20 mV in response to the optical stimulation. These two photoreactions were reversible and repetitive. Finally, electrical ABR measurements, which can assess function of the nervous system, ruled out the involvement of photoactivated glial ChR2 in the hearing loss. Taken together, because ICs' membrane potential strongly correlates with the EP, the change of the former underlay the reduction of the latter and thereby the impairment of hearing. This mouse model can represent short-term hearing fluctuation with the sudden onset and may contribute to translational medicine of SNHL.

Disclosures: **M.P. Sato:** None. **H. Taiga:** None. **H. Nin:** None. **G. Ogata:** None. **T. Yoshida:** None. **K. Hori:** None. **S. Komune:** None. **S. Uetsuka:** None. **C. Samuel:** None. **M. Masuda:** None. **T. Watabe:** None. **S. Kanzaki:** None. **K. Ogawa:** None. **H. Inohara:** None. **S. Sakamoto:** None. **H. Takebayashi:** None. **K.F. Tanaka:** None. **H. Hibino:** None.

Poster

710. Auditory System: Periphery

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Topic: D.05. Audition

Support: NIDCD DC004274 (HvG)

Title: Effect of temperature on exocytosis at the bullfrog hair cell ribbon synapse

Authors: *M. CHEN[1], H. VON GERSDORFF[2];
[2]Vollum Inst., [1]Oregon Hlth. and Sci. Univ., Portland, OR

Abstract: Synaptic transmission from the auditory hair cell to afferent fiber is a fundamental step in hearing. Synaptic release and endocytosis have been shown to be highly temperature sensitive in many preparations, but there is no study in amphibian hair cell ribbon synapse. To investigate the effect of temperature on exocytosis from hair cell ribbon synapse, we performed whole-cell patch-clamp recordings of Ca^{2+} current and exocytic membrane capacitance changes in semi-intact preparations of bullfrog hair cell and also recorded excitatory postsynaptic current (EPSC) of afferent fibers at room (22-24°C) and high (32-34°C) temperature. Temperature was adjusted by flow-heating with a temperature controller. Temperature was measured by a miniature thermistor close to the amphibian papillae that was recorded. An increase of temperature within this range increased Ca^{2+} influx of hair cells during 200-ms depolarization from -90 mV to -30 mV (e.g., Ca^{2+} current amplitude at room temperature: 604.5 ± 42.7 pA vs. high temperature: 756.6 ± 56.6 pA, n = 11, $p < 0.0001$, paired t test; Ca^{2+} charge transfer at room temperature: 80.8 ± 6.8 pC vs. high temperature: 109.0 ± 10.0 pC, $p = 0.0001$, n = 11). Activation of Ca^{2+} current was also accelerated by high temperature (Ca^{2+} current latency to peak at room temperature: 1.54 ± 0.09 ms vs. high temperature: 1.08 ± 0.12 ms, $p < 0.0001$, n = 11). Synaptic release triggered by 200-ms depolarization from -90 mV to -30 mV was enhanced at high temperature (ΔCm at room temperature: 84.8 ± 13.1 pF vs. high temperature: 136.2 ± 21.4 pF, $p = 0.0004$, n = 11). However, the increase of capacitance change triggered by depolarization was disproportionately to the increase in Ca^{2+} influx ($\Delta Cm/QCa^{2+}$ at room temperature: 1.06 ± 0.15 vs. high temperature: 1.25 ± 0.15, $p = 0.0236$, n = 11), indicating the release efficiency is increased at high temperature. We also recorded the EPSC of postsynaptic afferent fibers. We found that the amplitude and frequency of EPSC increased at high temperature. Furthermore, the high temperature also quickened the time to peak and fastened the decay of EPSC, which suggest that release is more synchronized at high temperature. Altogether, our results suggest that the increased synaptic release at high temperature is not only a result of increase of Ca^{2+} influx but could also involves other mechanisms, such as more readily releasable vesicles available at the active zone at high temperature, increased Ca^{2+} influx at high temperature induces more Ca^{2+} release from intracellular Ca^{2+} stores and enhances release efficiency.

Disclosures: M. Chen: None. **H. von Gersdorff:** None.

Poster

710. Auditory System: Periphery

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Support: NIH Grant R15DC013900

Washington State University start-up funds

Title: Berbamine derivatives protect against aminoglycoside induced hair cell damage in the zebrafish lateral line

Authors: *A. M. CAMINO[1], R. D. L. BONEY[1], B. E. BLOUGH[2], A. B. COFFIN[1]; [1]Washington State University-Vancouver, Vancouver, WA; [2]Res. Triangle Inst., Research Triangle Park, NC

Abstract: Our hearing depends on sensory hair cells that transduce mechanical stimulation into electrical stimuli, which are then perceived by the brain. When hair cells are damaged- from, aging, noise exposure and medications such as aminoglycoside antibiotics, hearing loss is often the result. Further, hearing loss affects approximately 360 million people globally. Although aminoglycosides are efficacious in treating bacterial infections in life threatening diseases such as cystic fibrosis and sepsis, they often result in the adverse side effect of hair cell damage. In fact, 20% of patients consuming these antibiotics develop some degree of hearing loss. To date, there are no FDA-approved drugs that prevent damage from aminoglycosides. Our goal is to develop a drug that prevents aminoglycoside-induced hearing loss, using a natural product as the chemical scaffold. Natural compounds represent a strong source of novel molecular scaffolds due to their enormous structural and chemical diversity, making them a rich fountainhead for drug leads. A previous study by our lab conducted a blind drug screen in the zebrafish lateral line and identified four natural compounds that share a modified quinolone scaffold and strongly protect hair cells by attenuating aminoglycoside uptake. The present study builds on this previous work, investigating analogs of our protective compounds to determine the core protective structure and the specific molecular targets. Analogs containing a phenyl functional group were found to robustly protect hair cells. Based on this data we now have promising lead compounds for further optimization to meet the therapeutic goal of developing a protective hearing loss drug candidate.

Disclosures: A.M. Camino: None. **R.D.L. Boney:** None. **B.E. Blough:** None. **A.B. Coffin:** None.

Poster

710. Auditory System: Periphery

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Title: Connexin 26 expression in superficial neuromasts of adult western mosquitofish gambusia affinis.

Authors: *N. MARTINEZ-RIVERA[1,2,3], J. L. SERRANO-VELEZ[2,4], I. I. TORRES-VAZQUEZ[2], H. GRIBBEN[5], E. ROSA-MOLINAR[6,2,3,7,8];
[1]Univ. of Kansas, Lawrence, KS; [2]Biol., Univ. of Puerto Rico-Rio Piedras, San Juan, PR; [3]Puerto Rico Ctr. for Envrn. Neuroscience, Inst. of Neurobiology, Univ. of Puerto Rico-Medical Sci., Old San Juan, PR; [4]Nikon Instruments Inc, Melville, NY; [5]Andor Tecnology, Inc, Belfast, United Kingdom; [6]Biol. Imaging Group, The Univ. of Kansas, Lawrence, KS; [7]Microscopy and Analytical Imaging Resources Core Laboratory, The Univ. of Kansas, Lawrence, KS; [8]The Eugene Bell Ctr. for Regenerative Biol. and Tissue Engineering, Marine Biol. Lab., Woods Hole, MA

Abstract: This study provides the first report of gap-junction proteins expressed in mechanosensory receptors of fishes. Prior work suggests that fast copulatory movements of the male Mosquitofish, *Gambusia affinis*, are controlled by a motor circuit containing mixed (chemical and electrical) synapses rather than chemical synapses only. A gap-junction protein, connexin 35, has been identified in dye-coupled motor neurons in spinal segments innervating skeletal muscles that move the gonopodium (a modified anal fin as functional copulatory organ) of adult male Mosquitofish. In our recent work characterizing the sensory circuit activating these motor neurons, we found positive immunolabeling of connexin 26, a gap-junction protein, in superficial neuromasts from the base of the anal fin of adult male and female Mosquitofish. Data analyses of male and female neuromast surface area and number of spots containing connexin 26 showed no significant differences. In addition, results showed that although the percent of relative frequency of spots was similar in males and females, females showed an increase in spot frequency in relation to the surface area. Finally, analysis showed a tendency of sexually dimorphic spatial distribution of connexin 26 within superficial neuromasts, with connexin 26 being more localized among female neuromasts than male neuromasts. Future research is focused on determining the spatial localization and functionality of these connexin 26-containing gap junctions in fish neuromasts.

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Poster

710. Auditory System: Periphery

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Program#/Poster#: 710.10/GG2

Topic: D.05. Audition

Support: R01 DC012552

McKnight Doctoral Fellowship Program

Title: The effect of glucose-6-phosphate dehydrogenase on cochlear and auditory function

Authors: *K. WHITE, M.-J. KIM, C. HAN, H.-J. PARK, Z. MENESES, L. GRANTGES, P. LINSER, S. SOMEYA;
Aging and Geriatric Research/ Speech, Language, and Hearing Sci., Univ. of Florida, Gainesville, FL

Abstract: Currently, there are no treatments or preventions of sensorineural hearing loss due to the lack of knowledge regarding the molecular mechanisms of this disorder. A large body of evidence suggests that oxidative stress plays a key role in progressive sensorineural hearing loss. Glucose-6-phosphate dehydrogenase (G6PD) is the first rate-limiting enzyme in the pentose phosphate pathway that converts glucose-6-phosphate into 6-phosphogluconate and $NADP^+$ into NADPH in the cytosol. Antioxidant enzymes such as glutathione reductase and thioredoxin reductase require NADPH in order to reduce oxidized glutathione to reduced glutathione and oxidized thioredoxin to reduced thioredoxin, that in turn protect cells from oxidative stress and cell death. **Therefore, we hypothesize that *G6pd* plays an important role in protecting cochlear cells from oxidative stress and maintaining hearing by supplying NADPH to the cytosolic antioxidant defense under normal conditions.** To investigate whether G6PD plays a key role in auditory function, we performed Auditory Brainstem Response (ABR) tests to measure hearing sensitivity in *G6pd* wild-type and *G6pd* deficient mice at 3-5 months of age. Our results showed no significant changes in ABR thresholds at 4, 8, 16, 32, 48 or 64 kHz between wild-type and *G6pd* deficient male or female mice at 3-5 months. To confirm these results, we counted the numbers of spiral ganglion neurons and hair cells in the cochlea from young wild-type and *G6pd* deficient males. There were no differences in the mean number of cochlear spiral ganglion neurons, inner hair cells or outer hair cells between wild-type and *G6pd* deficient mice. Taken together, these results suggest that *G6pd* deficiency does not affect cochlear histology or auditory function and that loss of *G6pd* is likely compensated by other NADPH-producing enzymes. We are currently investigating whether *G6pd* deficiency affects auditory function in middle-aged and old wild-type and *G6pd* deficient mice. Measurement of oxidative stress and antioxidant defense parameters are currently ongoing.

Disclosures: **K. White:** None. **M. Kim:** None. **C. Han:** None. **H. Park:** None. **Z. Meneses:** None. **L. Grantges:** None. **P. Linser:** None. **S. Someya:** None.

Poster

711. Auditory Processing: Spatial

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Topic: D.05. Audition

Support: NIH Grant DC007690

Title: Effect of direction-dependent envelope coding on the representation of sound location in the owl's midbrain

Authors: ***M. V. BECKERT**[1], B. J. FISCHER[2], J. L. PENA[1];
[1]Neurosci., Albert Einstein Col. of Med., Bronx, NY; [2]Mathematics, Seattle Univ., Seattle, WA

Abstract: Two primary features of sounds encoded by the auditory system are the sound's identity and location. We investigated how these two features are represented in parallel in the auditory midbrain of the barn owl, *Tyto alba*. In the brainstem of the barn owl the primary sound localization cues, interaural time (ITD) and level difference (ILD) are computed along two parallel pathways. It has been previously shown in our lab that the ILD pathway is better suited to encode sound identity by examining the locking of spikes to the sound's envelope across repeated trials of identical noise. We recorded neurons in the first midbrain structure that receives input from both of these pathways, the lateral shell of the inferior colliculus (ICls). We tested the ability of ICls neurons to lock to the envelop of repeated presentations of frozen noise by calculating the area under the curve of their shuffled autocorrelograms (SAC), which is a measurement of how reproducible is the spiking pattern for a given stimulus. We found that in ICls reproducibility is directly related to how far in space the sound is from the neuron's preferred sound direction. This is true for both ITD and ILD; however ILD appears to have a stronger influence on this direction-dependent representation of the envelope. We hypothesized that the direction-dependent reproducibility in ICls has an effect on the representation of sound location and identity in downstream structures by affecting population responses. To test this hypothesis we performed tetrode recordings in the optic tectum (OT), which contains a map of auditory space. We found that pairs of neighboring neurons are more likely to fire synchronously at their preferred direction and this synchrony drops off as a function of distance, thereby adding a temporal element to the representation. Finally, we tested whether this direction-dependent synchrony could influence the decoding of sound location and identity using a computational model. We found that synchrony can affect decoding of ambiguous stimuli by selectively

weighting neurons that respond to the dominant stimulus. In conclusion, we found a source of co-dependency between the encoding of sound identity and location through spike timing in the midbrain of the barn owl. This emphasizes how important spike timing can be, even in structures classically considered not to utilize a temporal code.

Disclosures: **M.V. Beckert:** None. **B.J. Fischer:** None. **J.L. Pena:** None.

Poster

711. Auditory Processing: Spatial

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Support: NIH, NIDCD Grant DC006262

Carls Foundation of Michigan

DOD Grant W81XWH-11-1-0267

Title: Dysfunctional myelin affects neural processing, neurochemistry and behavior

Authors: ***K. MAHERAS**[1], S. PERRINE[2], F. GHODDOUSSI[2], M. GALLOWAY[2], A. GOW[3,4,5];
[2]Psychiatry and Behavioral Neurosci., [3]Ctr. for Mol. Med. and Genet., [4]Carman And Ann Adams Dept. of Pediatrics, [5]Dept. of Neurol., [1]Wayne State Univ. Sch. of Med., Detroit, MI

Abstract: In the current study, we have generated mutant mice that lack Claudin 11 (Cldn11) in CNS myelin sheaths. In this compartment, Cldn11 forms tight junctions located along the outer and inner edges of the membrane spiral, preventing ions and small molecules from entering the intramyelinic space. The function of Cldn11 tight junctions is to improve the passive properties of the myelin membrane, by increasing membrane resistance and reducing capacitance, thereby boosting the speed of saltatory conduction. In the absence of Cldn11, myelin is dysfunctional and conduction velocity is slowed, most dramatically in small diameter myelinated fibers. Importantly, the absence of Cldn11 does not induce degenerative myelin pathology, enabling direct study of the impact of dysfunctional myelin on neuronal circuit properties such as neural processing.
Undoubtedly, slowed conduction velocity along myelinated axons increases temporal dispersion and, consequently, degrades information transfer between neural circuits. Herein, we explore the impact of dysfunctional myelin on neural processing in the conserved integration circuit of the auditory brainstem. We find that dysfunctional myelin perturbs neural processing and may

diminish the ability of mice to lateralize sound sources on the azimuth plane. Extrapolating this finding to higher order circuitry within the cortex, dysfunctional myelin may also disconnect distributed neural circuits across brain regions. Currently, we are functionally characterizing this type of behavioral deficit using auditory operant chambers.
Further investigation into the neural circuitry of the auditory brainstem reveals increased steady state levels of the neurotransmitters, glutamate and glutamine, which may exacerbate neural processing deficits or may be an attempt by neurons in the brainstem to compensate for increased temporal dispersion. In either case, our data are significant in two ways. First, they represent the first demonstration that non-degenerative changes in myelin membrane passive properties can lead to neurochemistry changes that perturb behavior/perception. Second, they have important implications for the etiology of behavioral disorders in general, and more specifically for the behavioral components of hypomyelinating and demyelinating diseases like multiple sclerosis.

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Poster

711. Auditory Processing: Spatial

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Topic: D.05. Audition

Support: Radboud Univ

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Title: sound localization performance to variations in spectral contrast.

Authors: *B. YOOSEFIZONOOZ, P. DE VETH, J. VAN OPSTAL;
Biophysics, Donders Cntr Neurosci, Radboud Univ., Nijmegen, Netherlands

Abstract: The acoustic spectral pinna cue-to-elevation mapping, is an ill-posed problem for the auditory system, as the spectral sensory input to the eardrum is always a convolution of the HRTF, H(f,E) (unknown) with the sound-source spectrum, X(f) (also unknown): S(f,E) = H(f,E)*X(f). The brain, therefore, should employ clever strategies to estimate source-elevation angle E. Our spectral correlation model may account for such a strategy with two prior assumptions: (1) HRTFs are unique, and (2) source spectra and HRTFs are uncorrelated: C[(X,H)]~0. Correlations between S(f,E) and all HRTFs are determined over a certain bandwidth, and elevation E is found at max[C(S,H)]. However, it is not well known which parts

of the acoustic spectrum dominate the mapping: the entire spectrum, vs. particular spectral bands? As the most prominent spectral cues lie within the 5-10 kHz band, we tested whether perhaps only this part of the spectrum matters. We systematically manipulated spectral contrast of broadband sounds in the 6-9 kHz band with the remaining frequencies (0.2-6 kHz and 9-20 kHz) over a 35 dB range (25 different spectra), and measured sound-localization performance of 8 participants over a wide range of locations in the 2D frontal hemifield. Our results indicate a systematic influence of spectral contrast on gain and bias of the stimulus-response relationships, suggesting that the elevation percept relies on the spectral content across the entire audible frequency domain.

Disclosures: **B. Yoosefizonooz:** None. **P. De Veth:** None. **J. Van Opstal:** None.

Poster

711. Auditory Processing: Spatial

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Title: Altered balance of excitation and inhibition in the auditory brain stem of Fragile X mice

Authors: E. MCCULLAGH[1], S. MINKOWICZ[2], M. HUNTSMAN[3], *A. KLUG[4];
[1]Physiol. and Biophysics, Univ. of Colorado Sch. of Med., Aurora, CO; [2]Florida Gulf Coast Universty, Fort Myers, FL; [3]Pharmaceut. Sci., [4]Physiol. & Biophysics, Univ. of Colorado, Aurora, CO

Abstract: Virtually all forms of autism involve some sort of auditory dysfunction, which points to the auditory system as one of the key systems involved in the physiology of autism. This autism related auditory dysfunction is most noticeable as impairments in communication skills, social difficulties and sound localization. Sound localization impairments include the ability to establish and separate between various competing sound sources, such as in a crowded noisy room. A recent theory posits that autism spectrum disorders may be caused by an imbalance in the ratio of excitation to inhibition, particularly in sensory systems.
Since sound localization relies heavily upon the synaptic ratio of excitation and inhibition (E/I ratio) in the auditory brainstem, we hypothesized that these neural circuits, may be altered in a mouse model of autism, the Fragile X mouse (Fmr1-/-).
We investigated differences in presynaptic and postsynaptic markers for the neurotransmitters glutamate, glycine and GABA in the nuclei of the mammalian sound localization pathway,

specifically the medial nucleus of the trapezoid body (MNTB) and the lateral superior olive (LSO). Both of these centers receive substantial excitatory and inhibitory inputs. Additionally, MNTB is a major source of inhibition to the sound localization pathway and projects to LSO. Recently it has been shown that there are more presynaptic GABAergic synapses in the MNTB of Fragile X mice (Fmr1-/-) as indicated by vesicular GABA transporter (VGAT). This study, however, did not investigate the effect of glycine, which, in the MNTB, becomes the primary inhibitory neurotransmitter in adulthood. The main reason why glycine has not been studied in the context of Fragile X is that the current E/I model of Fragile X assumes mostly changes occur in GABAergic inhibition. Here we address the role of glycine in Fmr1-/- mice by anatomical studies examining changes in number and organization of glycinergic neurons and synapses in the MNTB. Our results suggest that the glycinergic E/I ratio in Fmr1-/- mice is altered, suggesting that not only GABAergic but also glycinergic inhibition may play an important role in Fragile X.

Disclosures: **E. McCullagh:** None. **S. Minkowicz:** None. **M. Huntsman:** None. **A. Klug:** None.

Poster

711. Auditory Processing: Spatial

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 711.05/GG7

Topic: D.05. Audition

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Scientific Research Common Program of Beijing Commission of Education (KM201410025027)

Title: Processing of binaural cues conveyed by temporal fine structure and envelope in human auditory cortex

Authors: *Q. WANG[1], T. LI[1], G. LUAN[1], L. LI[2];
[1]Beijing Key Lab. of Epilepsy, Beijing Sanbo, Beijing City, China; [2]Dept. of Psychology and Beijing Key Lab. of Behavior and Mental Hlth., Peking University, Beijing, China

Abstract: In our auditory system, the peripheral system firstly filters broadband signals into narrowband waves, and then decomposes narrowband waves into quickly-varying temporal fine structures (TFSs) and slowly-varying envelopes. Whether TFS and envelope play critical roles in

music perception, sound localization, and speech recognition in complex environment has been under debated. Although previous neurophysiological studies discuss the temporal processing mechanisms in various levels of auditory system, how TFS and envelope are processing in auditory cortex is still unclear. With temporal fluctuations, both TFS and envelope could convey binaural cues, but the neural mechanisms of processing different binaural cues and their interactions in human auditory cortex is rarely discovered. In this study, using stereo-electroencephalographic (sEEG) with both highly temporal and spatial resolutions, we obtained electrophysiological data directly from primary auditory cortex (PAC) and non-primary auditory cortices (non-PAC) in epilepsy patients when they were listening to narrowband noises with different interaural correlations (IAC: +1, -1) and interaural time differences (ITD: 0,±0.2,±0.4,±0.6,±0.8 ms). The results showed that the TFS-sensitive responses (difference between IAC = +1 and IAC = -1) were shown in alpha-beta (8-13 Hz) band within PAC and non-PAC areas, but were shown in high-gamma (60-120 Hz) band only within non-PAC areas. On the other hand, both the PAC and non-PAC were sensitive to the ITD cues conveyed by both temporal fine structures and envelopes. PAC, but not non-PAC, showed a preference of the ITD sensitivity conveyed by envelope, with a significant correlation with the ITD sensitivity conveyed by TFS. Furthermore, a more robust correlation between temporal fine structure and envelope were shown in non-PAC. In conclusion, the binaural cues conveyed by TFS and envelope were processed hierarchically and built up gradually in human auditory cortex.

Disclosures: **Q. Wang:** None. **T. Li:** None. **G. Luan:** None. **L. Li:** None.

Poster

711. Auditory Processing: Spatial

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Title: Differential tuning of the low- and high-frequency components of the neurophonic spectrum reveals the spike contribution of barn owl's nucleus laminaris neurons.

Authors: *P. T. KUOKKANEN[1,2], A. KRAEMER[3], H. WAGNER[4], C. KOEPPL[5], C. E. CARR[3], R. KEMPTER[1,2];
[1]Inst. For Theoretical Biology, Humboldt-Universitaet Zu Berlin, Berlin, Germany; [2]Bernstein Ctr. for Computat. Neurosci., Berlin, Germany; [3]Dept. of Biol., Univ. of Maryland, College Park, MD; [4]Inst. for Biol. II, RWTH Aachen, Aachen, Germany; [5]Dept. of Neurosci., Carl von Ossietzky Univ. Oldenburg, Oldenburg, Germany

Abstract: In-vivo neural activity gives rise to trans-membrane currents that can be recorded as an extracellular field potential. These potentials are often challenging to interpret due to thousands of contributing sources. We aim at revealing the neural sources of the “neurophonic”. The neurophonic is a frequency-following extracellular potential that can be recorded in the network formed by the nucleus magnocellularis (NM) and the nucleus laminaris (NL) in the brainstem of the barn owl. NL anatomy is well understood, and putative generators of the neurophonic are the activity of afferent axons from NM, the synaptic activation onto NL neurons, and spikes of NL neurons.
We recorded the neurophonic in response to binaural high-frequency tones (3-7 kHz) close to the recording site's best frequency, and we varied the interaural time difference (ITD). The mean activity of the monaural inputs to NL does not change with ITD. However, their relative phase does, causing cancellation or summation of input signals. The activity of the binaurally-sensitive output of NL, i.e., firing rate of NL neurons, strongly depends on ITD. Our recordings contained both of these signals, and we analyzed the broad-band power spectrum of the response (0.1-8 kHz).
The low-frequency component (LFc, 200-700 Hz) of the neurophonic spectrum depended on ITD. The spectrum of extracellularly recorded NL neurons' action potentials closely resembled this component. Thus, the LFc reflects the contribution of action potentials initiated in NL neurons. The spectral component at the stimulus frequency (SFc) was much stronger than the LFc. The SFc also depended on ITD, reflecting the activity of the inputs and their relative phase change with ITD. The power spectrum at other frequencies did not depend on ITD.
We used the LFc as a proxy for NL neurons' local population activity, and the SFc as a proxy for NM axons' local population activity. We compared the ITD and frequency tunings of these proxies at each recording site. The best ITDs of the LFc and the SFc were independent. Also the tuning to stimulus frequency was different: LFcs showed typically a 400 Hz lower best frequency than SFcs. Both findings indicate that the LFc might originate from NL neurons' axons in the vicinity of the electrode. Related NL neurons can be located tens to hundreds of micrometers away. The findings are consistent with the known anatomy of NL. Our analysis thus reveals the small contribution of NL neurons to the neurophonic, improving our understanding of the extracellular field potential in the auditory brainstem.

Disclosures: **P.T. Kuokkanen:** None. **A. Kraemer:** None. **H. Wagner:** None. **C. Koeppl:** None. **C.E. Carr:** None. **R. Kempter:** None.

Poster

711. Auditory Processing: Spatial

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Program#/Poster#: 711.07/GG9

Topic: D.05. Audition

Support: Irish Research Council Government of Ireland Postgraduate Scholarship

Title: Decoding the cortical representation of acoustic space using EEG

Authors: *A. BEDNAR, E. C. LALOR;
Trinity Col. Dublin, Dublin, Ireland

Abstract: The human ability to localize sound is essential for monitoring the environment and helps us to analyze complex auditory scenes. Sound localization in the horizontal plane is mainly based on binaural acoustic cues: interaural time and level differences. Monaural (spectral) cues are crucial for sound localization in the vertical plane and when binaural cues are ambiguous. Although the acoustic cues mediating localization have been established behaviorally, it remains unknown how these cues are represented in human cortex. In particular, it is still a point of contention whether binaural and monaural cues are processed by the same or distinct cortical networks.

In this study, participants listened to a sequence of spatial sound stimuli while we recorded their neural activity using electroencephalography (EEG). The stimuli were presented over a loudspeaker array, which allowed us to deliver realistic, free-field stimuli in both the horizontal and vertical planes. Using a multivariate classification approach, we showed that it is possible to decode sound source azimuth and elevation from scalp-recorded EEG. Notably, the spatio-temporal pattern of EEG features that facilitated decoding differed based on the availability of binaural and monaural cues. In particular, we identified neural processing of binaural cues at around 120 ms post-stimulus and found that monaural cues are processed later at between 150 and 200 ms. Furthermore, different spatial activation patterns emerged for binaural and monaural cue processing. These spatio-temporal dissimilarities suggest the involvement of separate cortical mechanisms in monaural and binaural acoustic cue processing. In addition, our results did not reveal any differences in decoding performance between hemispheres, contradictory to the often reported right hemispheric dominance in sound localization.

The finding that we can decode sound source location using EEG has potential applications in the development of brain-computer interfaces, future hearing aids, and in measuring the fidelity of virtual acoustic environments.

Disclosures: **A. Bednar:** None. **E.C. Lalor:** None.

Poster

711. Auditory Processing: Spatial

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Topic: D.05. Audition

Support: NIDCD Grant DC014279

Pew Charitable Trusts

Title: Spatial encoding of speech in human auditory cortex

Authors: P. PATEL[1], L. K. LONG[1], J. HERRERO[2], A. D. MEHTA[2], *N. MESGARANI[1]; [1]Columbia Univ., New York, NY; [2]Feinstein Inst. for Med. Res., Manhasset, NY

Abstract: Sound localization is an important ability that allows an organism to monitor its surroundings. While the neural mechanisms of sound localization have been well characterized in nonhuman mammals, auditory spatial selectivity in human primary and non-primary brain areas has not been adequately characterized. Moreover, it remains unclear how the auditory cortex jointly encodes spatial cues with the spectrotemporal features of speech that carry linguistic and non-linguistic information. In this study, we used invasive neurophysiological recordings in humans while they listened to speech from five different directions to study the spatial selectivity in human auditory cortex. We found differential responses to spatial cues at the level of single electrodes in primary cortical areas, clearly revealing a spatially selective organization of responses. Moreover, a linear decoder was able to predict the direction of speech stimuli based on neural population data with accuracy as high as 80%. Furthermore, we characterized the joint encoding of spectrotemporal and spatial information. We found an orthogonal encoding of these cues, meaning that spectrotemporal feature selectivity was largely independent of the direction of the sound. Instead, we observed that the stimulus direction modulates the gain of the spectrotemporal tuning properties. These findings characterize the spatial encoding of speech sounds in the human auditory cortex, with implications for neurophysiological models of speech processing in the auditory pathway.

Disclosures: P. Patel: None. **L.K. Long:** None. **J. Herrero:** None. **A.D. Mehta:** None. **N. Mesgarani:** None.

Poster

711. Auditory Processing: Spatial

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Title: *In vivo* intracellular recordings from neurons of the lateral superior olive show narrow time windows of effective inhibition and high sensitivity to interaural time differences of transient sounds

Authors: *T. P. FRANKEN[1], P. H. SMITH[2], P. X. JORIS[1];
[1]KU Leuven, Leuven, Belgium; [2]Univ. of Wisconsin, Madison, WI

Abstract: The lateral superior olive (LSO) in the superior olivary complex contains binaural neurons that receive excitatory inputs from the ipsilateral ear relayed in the cochlear nucleus, and inhibitory inputs from the contralateral ear relayed in the medial nucleus of the trapezoid body. This interaction results in output sensitivity to interaural level differences (ILDs). This circuit also features large axosomatic synapses (such as the calyx of Held) and other specializations suggestive for timing, which are not easily reconciled with the proposed role of these neurons as ILD detectors. To study the computation performed by these neurons, we recorded from 29 cells in the LSO in the Mongolian gerbil using patch clamping in vivo. We presented monaural and binaural tones, at several ILDs, and monaural and binaural clicks, at several ILDs and interaural time differences (ITDs). We compared these responses to responses from medial superior olive (MSO) neurons. We labeled many of the LSO neurons with biocytin and studied the anatomy at the light and electron microscopic level. Our data represent the first retrieved neurons of the lateral superior olive that were recorded in vivo. Study of the soma location, somatodendritic anatomy and synaptic coverage allowed us to confirm the tonotopy of the lateral superior olive and classify the neurons as principal, type 5, marginal or multiplanar cells. The spontaneous intracellular activity showed EPSPs and IPSPs. All anatomical cell types received inhibition from the contralateral and excitation from the ipsilateral ear. We observed that, for simultaneous bilateral stimulation at the same sound level, contralaterally driven IPSPs often arrive earlier than ipsilateral EPSPs, even though they have to take a longer anatomical route through an extra

synapse. The responses to binaural clicks at different ITDs showed that the time window of effective inhibition is generally narrow and restricted to the initial, descending slope of the IPSP waveform. Click-ITD functions of LSO neurons showed steep ITD-sensitivity, as opposed to MSO neurons. We propose that inhibitory-excitatory interactions in the LSO, combined with the temporal specializations in the circuit, enable steep sensitivity to ITDs of transient sounds, unlike excitatory-excitatory interactions in the MSO.

Disclosures: **T.P. Franken:** None. **P.H. Smith:** None. **P.X. Joris:** None.

Poster

711. Auditory Processing: Spatial

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Program#/Poster#: 711.10/GG12

Topic: D.05. Audition

Title: The effect of spectral mismatch on binaural fusion in vocoder simulations of cochlear-implants for single-sided deafness

Authors: *J. WESS[1,2], J. G. W. BERNSTEIN[2];
[1]Univ. Of Maryland, College Park, MD; [2]Walter Reed Natl. Military Med. Ctr., Bethesda, MD

Abstract: Cochlear implants (CIs) can restore some degree of spatial hearing for individuals with single-sided deafness, SSD. However, there is a large amount variability in outcomes among those with SSD. One possible cause is spectral mismatch between the acoustic and electric ears. Standard frequency-to-electrode maps are programmed to provide the full speech bandwidth to a CI patient. This approach might be sub-optimal for a SSD-CI user, limiting the ability to fuse the very different auditory inputs from the two ears into a single fused sound. Fusion of signals across the ears is an integral step in enabling listeners to hear effectively in cocktail party environments. The goal of this study was to measure perceptual binaural fusion of speech stimuli. Vocoder simulations of SSD-CI listening were used to investigate the effect of spectral mismatch on subjective fusion in normal-hearing listeners. A virtual cocktail party was created by presenting combinations of one or more concurrent talkers to the left ear (normal unprocessed speech), to the right ear (vocoded speech) or to both ears (normal speech and vocoded speech). Each trial was 2 seconds long and the total number of concurrent talkers varied from one to six. The positions of individual electrodes along the cochlear array were simulated by adjusting the frequencies of the vocoder synthesis filters based on existing published data of average CI electrode insertion depth. In the “standard” condition, the analysis filters were set based on a standard clinical CI frequency allocation table. In the “place-matched” condition, the analysis filters were set to the same frequencies as the synthesis filters. After each trial, the

listener was asked how many total voices were heard. If listeners were able to perceptually fuse the voices that were presented to both ears, they should be more likely to report the correct number of total talkers in the scene. Conversely, if the signals were not fused between the ears, then listeners should report more total talkers then were actually presented. The results showed that listeners reported more total talkers when the vocoded speech was processed with a standard map then with the place-matched map. These data suggest that place-matched CI mapping has the potential to provide SSD-CI listeners with more opportunity for binaural fusion and subsequent improvements in spatial hearing. This paradigm provides a way to measure binaural fusion in a fast, straightforward way using speech signals representative of everyday listening environments. This method could be applied to the SSD-CI population to compare mapping strategies and outcomes after implantation.

Disclosures: J. Wess: None. **J.G.W. Bernstein:** None.

Poster

711. Auditory Processing: Spatial

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Topic: D.05. Audition

Support: NSF Grant IOS1010193

Title: Representing space in 3D: Response profiles of midbrain neurons in the free-flying bat

Authors: *N. B. KOTHARI, M. WOHLGEMUTH, C. F. MOSS;
Johns Hopkins Univ., Baltimore, MD

Abstract: Past research on the mammalian superior colliculus (SC) has elucidated its role in encoding 2D sensory space (azimuth and elevation) to guide species-specific orienting behaviors. In their natural environment, most animals, however, orient in 3D space, which requires sensory representation of not only the direction bat also the distance/depth of objects. Echolocating bats, for example, probe the environment with sonar signals and determine 3D object locations by processing acoustic information carried by echo returns. Here, we report on SC recordings from the free-flying echolocating big brown bat (*Eptesicus fuscus*) and present evidence demonstrating 3D spatial response areas in SC neurons. We used a 16 channel silicon probe to collect extracellular, single unit activity across laminae of the bat SC, using a telemetry system while the bat flew across an experimental room with objects positioned along its flight path. Synchronized high-speed audio and video recordings were used to quantify echolocation signals, head direction and flight trajectory data. From audio and 3D video position data, we built an

'echo model' to determine the arrival time and 3D location of acoustic stimuli arriving at the bat's ears. Combining the output of the echo model with synchronized neural recordings, we were able to reconstruct 3D spatial response profiles of SC neurons from free-flying echolocating bats. Importantly, multi-channel recordings across the dorsal-ventral axis of the SC allows us to investigate the relative timing of activity in sensory, sensorimotor and pre-motor layers. The results of this study reveal specializations for acoustic orienting by sonar and serve to advance a broader understanding of SC function in the context of species-specific orienting behaviors.

Disclosures: N.B. Kothari: None. **M. Wohlgemuth:** None. **C.F. Moss:** None.

Poster

712. Striate Cortex Circuits

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Program#/Poster#: 712.01/GG13

Topic: D.06. Vision

Support: NSERC

CIHR

Title: Laminar specific, orientation selective current source density response in cat area 18

Authors: M. Y. VILLENEUVE, P. KROPF, Z. YAO, V. M. MOCANU, *A. SHMUEL; McGill Univ., Montreal, QC, Canada

Abstract: Orientation selectivity shown by neurons in the visual cortex is considered to be the result of thalamo-cortical input and intra-cortical processing. Although much effort has been devoted to understanding the emergence of orientation selectivity, the exact mechanisms are still incompletely understood. In particular, it is still unclear how orientation specific signals are selectively relayed across cortical layers. Lamina-resolved current-source density (CSD) is hypothesized to mainly reflect synaptic activity. It should therefore be well suited to further our understanding of the role of intra-cortical signal processing in the emergence of orientation selectivity. Surprisingly, previous studies reported that the spatiotemporal CSD pattern is virtually invariant to the orientation of grating stimuli. Here we revisit this paradox by comparing 1-D laminar CSD in cat visual area 18 in response to gratings varying in contrast and orientation. Contrary to previous reports, we found a clear orientation selective component in the CSD responses. In particular, the primary sink in layer IV was found to extend from layer IV to supra-granular layers (Figure 1A, bottom, dark rectangle) when the preferred orientation (157.5

degrees), determined according to the rate of action potential response (Figure 1A, top), was presented. In contrast, no similar extension of the primary sink was observed in response to the non-optimal orientation (67.5 degrees; Figure 1A, bottom, red rectangle). We observed this orientation-dependent modification in the pattern of the CSD not only in response to high-contrast oriented grating (Figure 1A), but also at lower contrast (Figure 1B). This suggests that the observed effect is not due to a difference in overall amplitude of the neural activation but rather reflects differences in cortical signal processing. Hence, we present evidence that feature selection in early cortical processing can be achieved by selective information transfer between cortical laminae.

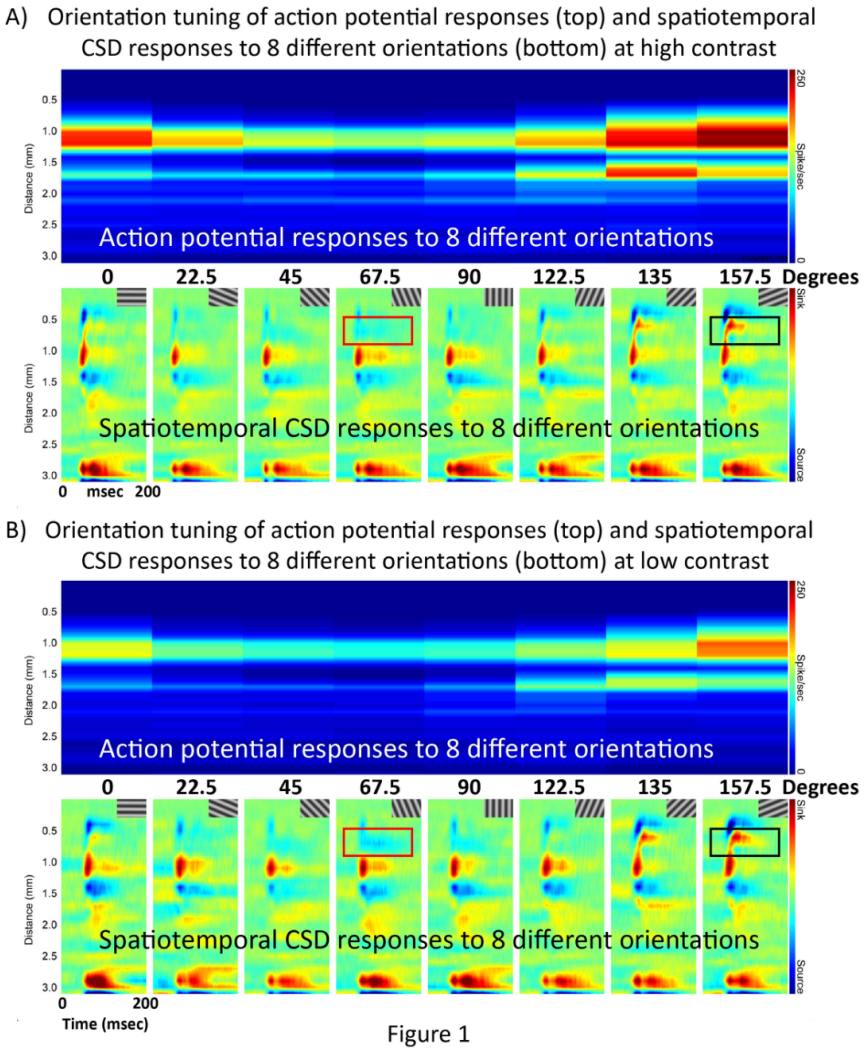

Figure 1

Disclosures: **M.Y. Villeneuve:** None. **P. Kropf:** None. **Z. Yao:** None. **V.M. Mocanu:** None. **A. Shmuel:** None.

Poster

712. Striate Cortex Circuits

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Program#/Poster#: 712.02/GG14

Topic: D.06. Vision

Support: EY019049

EY022478

Title: Spatial asymmetry and amplitude-dependent suppression: a novel mechanism for generating direction selectivity

Authors: *Y.-T. LI[1], Q. FANG[2], L. I. ZHANG[2], H. W. TAO[2];
[1]Caltech, Pasadena, CA; [2]USC, Los Angeles, CA

Abstract: Direction selectivity (DS) of neuronal responses is important for motion detection. Two models, spatially offset inhibition and temporally offset excitation, have been proposed for the generation of DS. While mouse primary visual cortex (V1) has become an important model, the mechanisms for DS there, in particular in the thalamo-recipient layer 4, remain obscure. In this study, we investigated this issue with in vivo whole-cell recordings and neuron modeling. For both membrane depolarization and excitatory synaptic responses, we found that the preferred direction and degree of direction tuning under moving-bar stimulation strongly correlated with the spatial skewness of input strengths evoked by stationary stimuli (flash bars) across the receptive field. Modeling revealed that this correlation cannot be accounted for by a simple linear summation of flash-bar evoked inputs. Sequential-bar stimulation revealed a forward suppression of the excitatory response to the second bar by the first bar, with the suppression level depending on the relative amplitude of the first-bar evoked response. By incorporating such amplitude-dependent suppression into our summation model, we could then successfully predict DS of the excitatory response under moving-bar stimulation. Our results suggest that DS in the mouse visual cortex can be generated via short-term plasticity on excitatory inputs whose strengths form an asymmetric spatial distribution. Such mechanism is distinct from those previously proposed in other species.

Disclosures: **Y. Li:** None. **Q. Fang:** None. **L.I. Zhang:** None. **H.W. Tao:** None.

Poster

712. Striate Cortex Circuits

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Program#/Poster#: 712.03/HH1

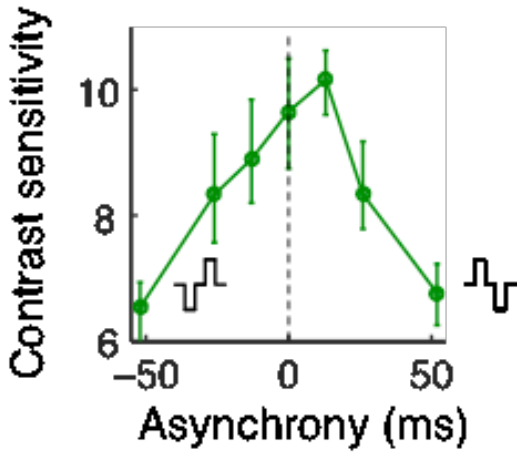
Topic: D.06. Vision

Support: Australian Research Council DP130102336

Title: Orientation discrimination depends on co-activation of on- and off-dominated visual channels

Authors: *A. W. FREEMAN[1], G. LUO-LI[1], D. ALAIS[2];
[1]Sch. of Med. Sci., Univ. of Sydney, Lidcombe, Australia; [2]Sch. of Psychology, Univ. of Sydney, Sydney, Australia

Abstract: Introduction. Visual responses to light increments and decrements are not symmetrical: both behavioural and neural data show that responses to darks are stronger and faster than to lights. Given the evidence that on- and off-centre subcortical inputs converge onto orientation-selective cortical neurons, we aimed to see whether the light/dark asymmetry influences orientation discrimination. **Methods.** We separated Gabor patches into light bars and dark bars, and presented the two components asynchronously. Bars were tilted 2° left or right of vertical but the tilt on any one trial was the same for light and dark bars. Adult human subjects indicated whether the tilt was leftward or rightward; response correctness and reaction time were both recorded. **Results.** The proportion of correct responses was measured as a function of stimulus contrast. Contrast sensitivity, calculated as the reciprocal of the contrast required to obtain 75% correct responses, was plotted against the asynchrony between light and dark bars. As shown in the figure, contrast sensitivity is optimal when light bars precede dark bars by 13 ms (one video frame), and the 95% confidence intervals show that this effect is significant. A similarly lop-sided tuning curve was found for reaction time. **Conclusion.** Given that on-pathways have a longer response latency than do off-pathways, this result suggests that orientation discrimination is optimal when on- and off-signals reach cortex at about the same time.



Disclosures: **A.W. Freeman:** None. **G. Luo-Li:** None. **D. Alais:** None.

Poster

712. Striate Cortex Circuits

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Topic: D.06. Vision

Support: Wellcome Trust 095668

Wellcome Trust 095669

Simons Collaboration on the Global Brain Research Award 325512

Title: Quantitative analysis of excitatory-inhibitory dynamics in the thalamocortical network of awake mice

Authors: ***I.-C. LIN**, M. OKUN, M. CARANDINI, K. D. HARRIS;
Univ. Col. London, London, United Kingdom

Abstract: While each region of cortex contains many cells, the bulk structure of local cortical activity can be summarized by a small number of "macroscopic variables", such as the summed activity of excitatory (E) and inhibitory (I) neuronal populations. It has been hypothesized that these macroscopic variables obey quantitative laws (e.g., the Wilson-Cowan equations), similarly to how statistical mechanics governs the macroscopic variables of physical systems.
To measure, manipulate, and model E-I dynamics, we combined large-scale electrophysiological recordings with independent optical stimulation of pyramidal (E) and parvalbumin-expressing

(*Pvalb*; I) neurons in primary visual cortex (V1) of quietly awake mice. The mice harbored a Thy18 allele (expressing ChR2 in a subpopulation of E neurons) and *Pvalb-Cre* allele, and expressed the red-shifted opsin C1V1(E122T/E162T) in *Pvalb* neurons via a conditional virus. E and I neurons were activated by brief blue (445 nm) and green (561 nm) laser pulses. Network activity at each moment was summarized by two variables: the total firing rates of all recorded wide-spiking (putative E) and narrow-spiking (putative I) cells.
A single blue pulse briefly activated the E population then the I population, followed by prolonged suppression of both populations, then a rebound in activity. Similarly, a green pulse increased the activity of the I (but not E) population, followed by shorter, less noticeable suppression of both populations. When pairs of pulses were presented to successively activate the two populations, the timing of the rebound after prolonged suppression was time-locked to the activation of E cells, rather than I cells.
To investigate potential mechanisms underlying this prolonged suppression, we recorded neural activity in the lateral geniculate nucleus (LGN) while activating cortical E/I populations. A blue pulse caused prolonged suppression in LGN, while a green pulse had little effect. These results suggest that the suppression generated by E activation involves a substantial thalamic contribution, while the suppression generated by I activation is due to the cortical network only. Moreover, as the suppression evoked by I activation exceeds that expected from synaptic $GABA_A$ activation alone, additional "slow" GABA components might be involved.
Finally, we asked whether these dynamics could be summarized by a set of equations. The Wilson-Cowan equations were insufficient, owing to their inability to predict prolonged suppression. We are currently developing a dynamical system model that includes variables representing slow GABA inhibition and LGN activity to summarise the observed population dynamics.

Disclosures: **I. Lin:** None. **M. Okun:** None. **M. Carandini:** None. **K.D. Harris:** None.

Poster

712. Striate Cortex Circuits

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Support: HFSP Fellowship

Society In Science-Branco Weiss Grant

Title: The affiliation of individual cortical neurons with global cortical networks

Authors: *K. CLANCY, I. ORSOLIC, T. MRSIC-FLOGEL;
Biozentrum, Univ. of Basel, Basel, Switzerland

Abstract: The neocortex is richly interconnected, and different cortical areas exhibit shifting affiliations with one another depending on behavioral state or context. This coupling is subserved in part by the excitatory long-range projections between cortical areas. Neighboring neurons may send projections to and receive input from local and distant areas. In addition to their heterogeneous connectivity, cortical neurons fall along a spectrum of engagement with shared local activity—while some are preferentially co-active with their neighbors, others are not functionally coupled to the local network. This response heterogeneity might reflect the variety of global influences on individual cells. We therefore undertook to map the relationship between the activity of individual neurons and the activity in distant areas, and sought to relate these functional maps to the strength of a cell's coupling to shared local activity.
In order to probe the affiliation of individual cells with distant networks, we recorded extracellularly from single units in visual cortex while simultaneously imaging cortex-wide activity in transgenic mice expressing the calcium indicator GCamp6s in CaMKII+ neurons. We made maps of the strength of correlation between individual units and various cortical areas. While many neurons shared similar cortex-wide correlation maps, a small proportion had unique affiliations, and these were often the cells most negatively coupled to local activity. Given that activity in primary sensory cortices not only reflects the occurrence of a sensory stimulus, but information about the animal's behavioral state and stimulus context, this method might be used to relate dynamic long range influences on the spiking of individual cortical neurons during different behaviors.

Disclosures: **K. Clancy:** None. **I. Orsolic:** None. **T. Mrsic-Flogel:** None.

Poster

712. Striate Cortex Circuits

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Support: R00 EY018407 (to JAC)

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Human Frontiers Science Program Fellowship (to MV)

Title: VIP interneurons mediate cortical phenotypes in a genetic model of schizophrenia

Authors: *M. VINCK, R. BATISTA-BRITO, K. FERGUSON, J. A. CARDIN;
Yale Univ., New Haven, CT

Abstract: Schizophrenia is associated with altered cognitive and perceptual processing, as well as dysregulation of normal brain rhythms such as gamma oscillations (30-80Hz). Current evidence suggests that dysregulation of GABAergic interneurons contributes to neural and behavioral deficits in this disease. However, the roles of diverse interneuron populations in psychiatric disease remain poorly explored. Neuregulin 1 (*NRG1*) and its interneuron-specific receptor *ERBB4* are risk genes for schizophrenia. Using a conditional ErbB4 deletion model, we used awake behaving animals to test the role of distinct interneuron populations in disease-related deficits. In contrast to the prevailing view, which supports a role for parvalbumin (PV)-expressing interneurons in the pathophysiology of schizophrenia, we find that deletion of ErbB4 specifically from vasoactive intestinal peptide (VIP)-expressing interneurons mediates many observed phenotypes. ErbB4 removal from VIP interneurons during development leads to changes in their activity, along with elevated cortical firing rates and severe dysregulation of the fine temporal organization of excitatory and inhibitory activity patterns. ErbB4 deletion from VIP interneurons abolishes the normal behavioral state-dependence of excitatory cortical activity. As a result of these alterations, animals in which VIP interneurons lack ErbB4 exhibit reduced visual cortical responses and impaired learning on a visual task. Our data support novel roles for VIP interneurons in cortical circuit development and the pathophysiology of schizophrenia, and provide a new perspective on the disruption of cortical circuit function in this complex disease.

Disclosures: **M. Vinck:** None. **R. Batista-Brito:** None. **K. Ferguson:** None. **J.A. Cardin:** None.

Poster

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Topic: D.06. Vision

Support: NSF GRFP DGE-1321851

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Title: Inhibition and synaptic integration in simple cell receptive fields in cat V1

Authors: *M. M. TAYLOR, L. A. PALMER, D. CONTRERAS;
Neurosci., Univ. of Pennsylvania Perelman Sch. of Med., Philadelphia, PA

Abstract: Simple cells in primary visual cortex (V1) are sensitive to the orientation of visual stimuli. This property arises because their receptive fields (RFs) consist of side by side elongated subregions of opposite contrast preference. A bright bar presented in a bright subregion (sign-matched) drives the cells output while presented in a dark subregion (sign-mismatched) suppresses output. We found that sign-matched stimuli evoke not only an excitatory synaptic conductance but also an inhibitory conductance. We termed this "sign-matched inhibition" to distinguish it from the expected and established inhibition triggered by a sign-mismatched stimulus (that we call "sign-mismatched inhibition"). We find that sign-matched inhibition is comparable in magnitude to sign-mismatched inhibition. Therefore, inhibition is widespread in space across the RF.

Do both types of inhibition serve the same function? We test the role of sign-mismatched inhibition by pairing a sign-matched stimulus with sign-mismatched stimulus while recording intracellularly from simple cells in vivo. We examine the time-dependent properties of combinations of sign-matched and sign-mismatched inhibition. For three delays between the two bar onsets (-8, 0, and 8 ms), we examine the peak amplitude of the evoked PSP and the dV/dt of the PSP onset. Inhibition from a sign-mismatched stimulus reduces the amplitude and dV/dt of the excitatory response to a sign-matched stimulus, when the sign-mismatched bar is presented before or concurrently with the sign-matched bar. This suggests that the onset timing of sign-matched and sign-mismatched inhibition is not the same, which in turn suggests two functionally distinct cellular sources of inhibition. Surprisingly, we also find in a subset (~30%) of simple cells, presentation of a sign-mismatched bar had no effect on the PSP evoked by the sign-matched bar, indicating that a sign-mismatched stimulus does not always evoke an inhibitory response.

Our results suggest that while inhibition concurrent with an excitatory response limits its time course, inhibition triggered from an inhibitory response (sign-mismatched) contributes to the spatial integration of inputs within the RF of simple cells.

Disclosures: **M.M. Taylor:** None. **L.A. Palmer:** None. **D. Contreras:** None.

Poster

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Topic: D.06. Vision

Title: Dopamine elicits lamina- and frequency-specific increase of information in the local-field-potentials of the macaque V1

Authors: *D. ZALDIVAR[1], J. GOENSE[2], S. LOWE[3], N. LOGOTHETIS[4], S. PANZERI[5]; [1]Physiol. of Cognitive Processes, Max Planck Inst. For Biol. Cybernetics, Tuebingen, Germany; [2]Inst. of Neurosci. and Psychology, Univ. of Glasgow, Glasgow, United Kingdom; [3]Sch. of Informatics, Inst. of Adaptive and Neural Computation, Edinburgh, United Kingdom; [4]Max Planck Inst. for Biol. Cybernetics, Tuebingen, Germany; [5]Inst. Italiano di Tecnologia, Rovereto, Italy

Abstract: Purpose: Local field potentials (LFPs) reflect the aggregate activity of neural populations generated by different neural mechanisms and expressed in different frequency domains. Each frequency range reflects, at least in part, different aspects of neural activity and capture the activity expressed by different processing pathways[1]. In particular, previous studies showed the activity reflected in the low (< 20 Hz) and high-frequencies (> 50 Hz) dissociate from the activity of the middle-frequency band (18 – 38 Hz). It has been proposed, based on statistical considerations, that this middle frequency band reflects the influence of neuromodulation pathways[1]. However, it is not know whether and how this middle-frequency band reflects neuromodulation and whether it relates to stimulus encoding.
Methods: We recorded LFPs in four anesthetized non-human primates (*macaca mulatta*), during spontaneous activity and presentation of movie clips, using 16-contact laminar probes (NeuroNexus) on a single shank of 3 mm long (50 µm thick) and with electrode-sites spaced 150 µm apart spanning the entire cortical depth of V1. We pharmacologically mimicked dopaminergic (DAergic) neuromodulation, by systemically applying L-DOPA+Carbidopa. L-DOPA is metabolic precursor of dopamine (DA) and once it crosses the blood-brain-barrier, is immediately metabolized into DA[2].
Results and Conclusions: DAergic neuromodulation elicited frequency- and stimulus dependent power changes in the recorded LFPs. During spontaneous activity, we observed a remarkable increase specific to the middle-frequency (18 – 38 Hz) band power accompanied by a decrease of gamma (50 – 150 Hz) power. In contrast, during visual stimulation with movie clips DA increased both the power of gamma and of the middle frequency band. Moreover, DA increased the information in LFP power, particularly superficial and deep layers and in the gamma (50 – 100 Hz) frequency band. Overall, our results show that the middle-frequency band captures endogenous non-stimulus driven oscillations that are modulated by dopamine, and that dopamine regulates gamma-range information coding in visual cortex.

References

1. Belitski, A.*, et al.* Low-frequency local field potentials and spikes in primary visual cortex convey independent visual information. *J Neurosci* 28, 5696-5709 (2008).
2. Zaldivar, D., Rauch, A., Whittingstall, K., Logothetis, N.K. & Goense, J. Dopamine-induced dissociation of BOLD and neural activity in macaque visual cortex. *Curr Biol* 24, 2805-2811 (2014).

Disclosures: **D. Zaldivar:** None. **J. Goense:** None. **S. Lowe:** None. **N. Logothetis:** None. **S. Panzeri:** None.

Poster

712. Striate Cortex Circuits

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Topic: D.06. Vision

Support: This work was funded by NHMRC Project grants APP1008287 and APP1066588, and a Human Frontier Science Program Career Development Award to Nicholas Price.

Title: Faster responses under low contrast stimulation in rat primary visual cortex

Authors: ***M. GHODRATI**[1,2,3], D. S. ALWIS[1,2,3], N. S. C. PRICE[1,2,3];
[1]Dept. of Physiol., Monash Univ., Melbourne, Australia; [2]Neurosci. Program, Biomedicine Discovery Inst., Melbourne, Australia; [3]ARC Ctr. of Excellence for Integrative Brain Function, Monash Univ. Node, Monash Univ., Melbourne, Australia

Abstract: Orientation selectivity, the hallmark property of neurons in mammalian primary visual cortex (V1), is invariant to stimulus luminance and contrast, which are two basic properties of visual stimuli in the natural world. Under low light or contrast levels (e.g., in fog), driving and games involving hand-eye co-ordination become more difficult. We wondered how the time-course of neuronal orientation processing was affected by luminance and contrast, and whether it could account for the observed perceptual effects. Here, we characterised the dynamics of orientation selectivity in V1 neurons (N=286) of halothane-anaesthetized rats, using a reverse correlation method. Stimuli comprised a sequence of sinusoidal gratings (each visible for 33 ms) with continually changing orientation, phase, and spatial frequency. We varied luminance and contrast in four different stimulus blocks: 1) high contrast; 2) low contrast; 3) high luminance; 4) low luminance. Averaged across all conditions, orientation selectivity was evident from 60±23 ms, with peak selectivity at 80±20 ms post stimulus onset. Consistent with previous reports using forward correlation, orientation selectivity did not vary with luminance contrast, and we now show that the mutual information between neuronal activity and stimulus orientation is the same under all conditions. Although neurons responded earlier with higher luminance stimuli, the response latency to high-contrast gratings was surprisingly longer than to low-contrast stimuli. Based on our computer simulations, we suggest that the longer latencies in high-contrast conditions are due to membrane hyperpolarization after prolonged exposure to high-contrast stimuli. The hyperpolarization at high-contrast may increase signal-to-noise ratios while a more depolarized membrane under low-contrast may lead to greater sensitivity to weak stimuli.

Disclosures: **M. Ghodrati:** None. **D.S. Alwis:** None. **N.S.C. Price:** None.

Poster

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Support: Human Frontier Science Program – CDA00029

National Health and Medical Research Council – APP1066588

Title: The neuronal and perceptual effects of visual masking

Authors: ***K. L. RICHARDS**[1], D. S. ALWIS[2], E. ARABZADEH[3], N. S. C. PRICE[4]; [1]Clayton, Australia; [2]Florey Inst., Melbourne, Australia; [3]Australian Natl. Univ., Canberra, Australia; [4]Physiol., Monash Univ., Melbourne, Australia

Abstract: The perception and neural representation of a stimulus are influenced by other stimuli that occur nearby in space or time. The phenomenon of visual masking describes the reduction in the perception of a target stimulus by a preceding (forward masking) or succeeding (backward masking) stimulus. In this way, masking illustrates a disconnect between the physical stimulus, its neuronal representation, and its percept. To understand the neural correlates of masking, we examined how visual masks affect orientation discrimination in awake rodents, and separately examined neuronal responses to similar stimuli in the primary visual cortex (V1). Long-Evans rats (n=6) were trained to discriminate the orientation of a Gabor patch presented for 42 ms. These target stimuli were presented at stimulus onset asynchronies (SOA) of –250 to 250 ms relative to an uninformative mask. In a separate cohort of halothane-anaesthetised animals (n=28) neuronal responses to target and mask stimuli were recorded from all layers of V1 using a 32-channel linear array. Early and late components of the neuronal response were analyzed separately. Behaviourally, for both forward and backward masking conditions, discrimination performance was impaired when the target and mask occurred close in time (i.e. short SOAs). Similarly, across all cortical layers, neuronal firing rate and orientation selectivity decreased monotonically with shorter SOAs. However, within early and late response components the reductions in orientation selectivity were not reliably predicted by changes in firing rate. Collectively, our data suggests that changes in V1 activity are responsible for the impairment in perceptual discrimination during masking. Based on the weak, non-selective responses to our mask stimuli, we suggest that both forward and backward visual masking may develop throughout the visual processing hierarchy through cumulative normalization computations.

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Poster

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Title: Correlations of interneuron populations with Pyramidal cells in mouse primary visual cortex

Authors: ***M. DIPOPPA**, M. CARANDINI, K. D. HARRIS;
Univ. Col. London, London, United Kingdom

Abstract: Different classes of GABAergic interneuron are thought to play different roles in cortical function. Interneurons expressing Parvalbumin (Pvalb) are thought to provide inhibition that is proportional to local excitation (Xue et al., Nature 2014). Interneurons expressing somatostatin (*Sst*) are thought to integrate excitatory activity over larger regions and inhibit Pyramidal (Pyr) neurons, playing a role in computations such as surround suppression (Adesnik et al., Nature 2012). Interneurons expressing vasoactive intestinal peptide (*Vip*) are thought to disinhibit Pyr cells via their strong inhibition of *Sst* cells (Fu et al., Cell 2014).
To test these proposal, we used 2-photon imaging to measure the activity of these interneuron types together with Pyr cells, in layers 2/3 of V1 of head-fixed mice that were free to run while viewing drifting gratings of various sizes. We identified interneuron types in transgenic mice expressing tdTomato in *Sst*, *Vip*, or *Pvalb* neurons, and measured Ca^{2+}-related fluorescence with unconditional viral expression of GCaMP6m. We identified putative Pyr by the sparseness of their neural activity.
The three interneuron types showed different relationships to Pyr activity. The *Pvalb* population had strong positive spontaneous correlations with the Pyr population, in running or stationarity. *Vip* cells showed similar behavior to *Pvalb* cells, but their correlations with Pyr cells were less prominent in stationarity, when their firing was overall weaker. The *Sst* population had negative correlations with Pyr cells during running, but these were also substantially weakened in

stationarity. Noise correlations showed similar structure to spontaneous correlations.
Signal correlations revealed a more complex picture. For small stimuli (between 5 and 30 deg), signal correlations showed a similar organization to spontaneous and noise correlations. For larger stimuli, however, signal correlations were less reliable between experiments, with *Sst* cells often showing positive correlations with Pyr activity.
These results indicate that the nature and even the sign of interneuron-Pyr correlations strongly depend on sensory and behavioral context. The predictions of the disynaptic inhibitory circuit involving *Vip* and *Sst* cells appear to be correct only for some of these sensory and behavioral contexts.

Disclosures: **M. Dipoppa:** None. **M. Carandini:** None. **K.D. Harris:** None.

Poster

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Title: Estimating simple and complex cell properties using a convolutional model with parameterized rectifiers

Authors: P. M. T. NGUYEN, *C. L. BAKER;
Ophthalmology, McGill Vision Res. Unit, Montreal, QC, Canada

Abstract: Neurons in the early visual cortex have often been classified into two types based on their receptive field (RF) properties. Simple cells have RFs with segregated excitatory and inhibitory zones, and phase-dependent responses (F1/F0 ratio > 1) to sinewave gratings. Complex cells' RFs have mixed excitatory and inhibitory responses, with F1/F0 < 1. The behaviour of simple cells can be understood in terms of linear-nonlinear (LN) models, and readily estimated with simple system identification methods such as spike-triggered averaging. Due to the phase invariance, complex cells are much harder to estimate, requiring heavily parameterized models.
Here we present a new 2-layer convolutional neural network model, with a parameterised rectifier between layers, which allows us to capture the responses of both simple and complex cells with only a single additional parameter, "alpha". The optimization of model parameters is

achieved through a backpropagation algorithm using gradient descent.
We recorded the extracellular single-unit responses of neurons in Areas 17 and 18 of anesthetised, paralyzed cats using 32-channel multielectrodes. Natural image stimuli were used to estimate and predict the neurons' responses, while sinewave gratings were used to estimate F1/F0 ratios. We collected neuronal responses to separate sets of stimuli. One dataset was used for model estimation, and a separate holdback dataset was used solely to score the performance of the estimated model. Model performance was measured as the "variance accounted for" (VAF) in the model's prediction of the holdback dataset.
The convolutional network method yields higher VAFs than previous methods of receptive field estimation, such as spike-triggered average and spike-triggered covariance, for both simple and complex cells. Furthermore we show that the alpha parameter is predictive of a neuron's F1/F0 ratio. Also we show how the parameterised rectifier unit may be used to estimate the degree of push-pull imbalance between excitatory and inhibitory inputs.
This work suggests that convolutional neural networks may be a fruitful approach to characterizing receptive fields of early visual cortex cells.

Disclosures: P.M.T. Nguyen: None. **C.L. Baker:** None.

Poster

712. Striate Cortex Circuits

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Topic: D.06. Vision

Support: Paul G. Allen

Title: An extended retinotopic map of mouse cortex

Authors: *J. ZHUANG[1], L. NG[2], D. WILLIAMS[3], M. VALLEY[1], Y. LI[2], M. GARRETT[1], J. WATERS[1];
[1]Neural Coding Dept., [2]Technol. Dept., [3]Manufacturing Engin. Dept., Allen Inst. for Brain Sci., Seattle, WA

Abstract: Visual perception and behavior are mediated by a network of cortical areas that have been distinguished using architectonic and retinotopic criteria. We employed fluorescence imaging and GCaMP6 reporter mice to generate retinotopic maps of visual areas in the mouse. Changes in GCaMP6 fluorescence were >2 orders of magnitude larger and 5 times faster than intrinsic optical signals, the standard imaging technique for mapping visual areas. The resulting high signal-to-noise map includes new retinotopically-organized regions medial and anterior to

known visual areas. To compare the borders of cortical areas identified using different techniques, we registered functional and projection-based retinotopic maps to borders identified with chemo- and cytoarchitectonic markers. This comparison revealed that retinotopic organization extends into barrel cortex and retrosplenial cortex and that the locations of borders between visual areas identified with different techniques differed by several hundred micrometers, which is on the order of size of some of the smaller visual areas. We also assessed the representation of visual space within each visual area, showing that four higher visual areas bordering V1 (LM, P, PM and RL) display complementary representations, with overlap primarily at the central hemifield. Our results extend our understanding of the organization of mouse visual cortex to include up to 16 retinotopically distinct maps. To identify the sources of wide field signal, further experiment will align wide field retinotopic maps with receptive field locations of cellular structures recorded by 2-photon imaging.

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Poster

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Topic: D.06. Vision

Support: Allen Institute founders, Paul G. Allen and Jody Allen

Title: Inactivation mapping of mouse cortex during a visual detection task

Authors: ***M. VALLEY**, D. R. OLLERENSHAW, J. MILES, S. OLSEN, J. WATERS; Allen Inst. for Brain Sci., Seattle, WA

Abstract: The degree to which the mouse cortex is necessary for performing highly-trained and repetitive laboratory behaviors remains controversial. Cortical lesions have been shown to result in little or no decrement in behavioral performance in a variety of sensory modalities. However, recent work using temporally-focused optogenetic lesions have renewed interest in defining exactly how cortical circuits participate in trained behaviors. Here, we systematically inactivate regions of cortex in VGAT-ChR2 mice during performance of a visual detection task. Our procedure allows for arbitrary targeting of cortical regions using guidance relative to retinotopic maps, intrinsic stimulus maps, and stereotaxic coordinates. Using targeted inactivation in a variety of cortical locations, we map the involvement of these regions in visual detection, lick-

based behavioral report, locomotion, and in the modulation of arousal as measured by pupillary diameter.

Disclosures: **M. Valley:** None. **D.R. Ollerenshaw:** None. **J. Miles:** None. **S. Olsen:** None. **J. Waters:** None.

Poster

712. Striate Cortex Circuits

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Topic: D.06. Vision

Title: Mesoscale cortical dynamics measured with wide-field calcium imaging during a visual detection task in mice

Authors: ***D. R. OLLERENSHAW**[1], M. T. VALLEY[2], J. ZHUANG[2], N. H. CAIN[2], P. A. GROBLEWSKI[2], M. E. GARRETT[2], B. DANSKIN[2], J. WATERS[2], S. R. OLSEN[2]; [1]Allen Inst. For Brain Sci., Seattle, WA; [2]Allen Inst. for Brain Sci., Seattle, WA

Abstract: Sensory guided behaviors involve a dynamic interplay of many distributed cortical areas to process an external stimulus and choose an appropriate motor response. Here, we characterize mesoscale cortical activity in mice engaged in a visually guided detection task. We use wide-field imaging of transgenic mice expressing the calcium indicator GCaMP6 in genetically defined cell populations. Our imaging preparation permits the simultaneous measurement of population activity from multiple visual areas through a cranial window or multiple brain regions across the entire dorsal surface of the cortex through the intact skull. In the behavioral task, head-fixed mice are trained to respond to briefly flashed visual stimuli with contrasts spanning the detection range. Using traditional signal detection theory metrics, we describe the coding of stimulus information in V1 and higher visual areas. We extend this analysis across the dorsal surface of the cortex to map a distributed network of cortical regions that predict the animal's behavioral choice at the contrast detection threshold. These experiments help to further elucidate the cortical network involved in a sensory task, while also provide a map for targeting future spatially and temporally restricted optogenetic perturbation experiments.

Disclosures: **D.R. Ollerenshaw:** None. **M.T. Valley:** None. **J. Zhuang:** None. **N.H. Cain:** None. **P.A. Groblewski:** None. **M.E. Garrett:** None. **B. Danskin:** None. **J. Waters:** None. **S.R. Olsen:** None.

Poster

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Support: PFV/10/008, Fonds voor Wetenschappelijk onderzoek, ERC Stg-260607

Title: Functional magnetic resonance imaging (fMRI) - guided single unit recordings reveal first order disparity selectivity in area PIP of the macaque brain.

Authors: *A. ALIZADEH[1], P. JANSSEN[2];
[1]Katholieke Univ. Leuven, Leuven, Belgium; [2]KU Leuven, Leuven, Belgium

Abstract: Binocular disparity provides a strong, unambiguous cue for depth perception in primates. Several cortical areas in both the dorsal and the ventral visual pathways are involved in the processing of depth from binocular disparity. Previous functional magnetic resonance imaging (fMRI) studies in monkeys (Van Dromme et .al, PloS Biol, 2016) revealed stronger activations in the medial bank of the caudal intraparietal sulcus (IPS, area PIP) in response to higher-order (curved and slanted) disparity stimuli compared to zero-order (flat) stimuli. The current study was carried out to investigate the higher-order disparity selectivity in area PIP at the single-cell level. The search test consisted of 32 second-order stimuli in addition to 8 first-order stimuli (2 vertical and 2 horizontal linear disparity gradients, square shape, 4 of which were 8.4° and the other 4 were 18.75° in diameter). We tested for both second- and first-order disparity selectivity (including monocular controls) and selective cells were additionally tested with preferred and non-preferred stimuli at 5 different positions in depth (mean disparity from -0.5 to 0.5). In total, we recorded from 207 PIP neurons showing significant visual responses to the stimuli of the search test presented at the fixation point in 3 monkeys. A large fraction of all visually-responsive neurons (87 neurons, 42%) exhibited significant selectivity for first- or second-order stimuli which could not be explained by the monocular responses. More than half of these selective neurons (43/79, 54%) preserved their selectivity across positions in depth when tested with first-order (slanted planar) stimuli. However, contrary to the predictions of the previous fMRI studies, none of the PIP neurons tested (N=56) maintained its selectivity across different positions in depth for second-order (curved) stimuli. The average response to the preferred second-order stimuli at three positions in depth (mean disparity -0.25 to +.25) was significantly larger than the response to zero-order stimuli at the same positions in depth, which could explain the fMRI activations. In general, PIP neurons preserved their 3D selectivity across different stimulus sizes (ranging from 3.1° to 18.8°). Disparity-selective PIP neurons usually had large receptive fields in the contralateral hemifield, frequently including the fovea. Thus, the

fMRI activation elicited by curved surfaces in area PIP is primarily based on a neural selectivity for first-order stimuli.

Disclosures: **A. Alizadeh:** None. **P. Janssen:** None.

Poster

713. Visual Cortex Dynamic Properties

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Title: Dynamics of "population coupling" between synaptic inputs and local cortical activity during visual processing

Authors: ***N. WRIGHT**, M. S. HOSEINI, R. WESSEL;
Physics, Washington Univ. In St. Louis, Saint Louis, MO

Abstract: The spiking activity of a cortical neuron is coordinated with that of its neighbors. This "population coupling" has significant implications for sensory coding, and is thought to represent the effects of both anatomical and emergent network properties on cortical function. Here, we investigate two questions vital to a better understanding of cortical sensory processing. (1) How strongly-coupled are the synaptic inputs (g) to a neuron with the local population activity (LFP), and how does this change with sensory stimulation? (2) To what degree are the dynamics of g-LFP coupling determined by the network, and what are the relevant network parameters?
To address the first question, we simultaneously recorded the membrane potential (V) and the nearby LFP in the visual cortex of the turtle eye-attached wholebrain *ex vivo* preparation, during ongoing and visually-evoked activity. We then applied a recently-developed algorithm to infer g from V, and calculated the g-LFP coupling. We found that during ongoing activity, g-LFP coupling differed from neuron to neuron. Moreover, coupling during (the highly-variable) evoked activity was not static, but transiently increased following stimulation, before relaxing to intermediate values. Comparison with a parallel study suggests coupling falls off very gradually with cortical distance. That is, global fluctuations strongly influence the sensory-evoked inputs to individual neurons.
To address the second question, we implemented a small-world network of leaky integrate-and-fire neurons, subject to Poisson external inputs and synaptic depression with recovery. This

model reproduces two experimentally-observed aspects of evoked activity: large across-trial response variability, and the g-LFP coupling dynamic. Importantly, it does so despite the fact that external inputs are uncorrelated across pairs of neurons. Instead, these response properties are largely determined by the network state, which is highly sensitive to such network parameters as spatial clustering, synaptic time constants, and adaptation. We are currently investigating the ability of the model to reproduce other experimental results.
Together, our results provide a clearer picture of the subthreshold coordination dynamics corresponding to suprathreshold population coupling in cortex. Moreover, they implicate specific network properties that shape cortical coordination during sensory processing.

Disclosures: **N. Wright:** None. **M.S. Hoseini:** None. **R. Wessel:** None.

Poster

713. Visual Cortex Dynamic Properties

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Title: Evaluating network criticality with membrane potentials

Authors: *J. K. JOHNSON[1], N. C. WRIGHT[2], R. WESSEL[2];
[1]Washington Univ. Physics, University City, MO; [2]Physics, Washington Univ., Saint Louis, MO

Abstract: Membrane potentials (Vm) are a well-established observable in neural systems that are also used for carrying information by the brain itself. Recordings from intact functional networks are becoming increasingly accessible with recent advances in experimental automation and in-vivo techniques. Subthreshold Vm fluctuations are primarily driven by synaptic inputs via the dendritic arbor of a neuron. This constitutes a subsampling of the spiking activity of the network to which that neuron belongs, with the caveat that such post-synaptic-potentials are non-linear transformations**.** Therefore, it makes sense to test the extent to which Vm fluctuations can contain information about network variables. We addressed this with a combined experimental and modeling approach. We recorded ongoing activity from pyramidal neurons in turtle visual cortex, and found that distributions of subthreshold fluctuations displayed size and duration power laws with a scaling relation consistent with the critical point of a second-order phase transition. Our modeling results suggest that we can expect analysis of neural inputs to be

consistent with results that are normally attained via multi-electrode array recordings or calcium-fluorescent imaging. Values from input fluctuations were found to be systematically offset from network values and also to co-vary with changes in connectivity and inhibition that affect network state. This has never previously been demonstrated using single-unit recordings of any kind. Thus this work is important for two reasons. First it expands the tools available to researchers looking for behavior or other properties concomitant with a near-critical state (presumably emergent properties suggestive of self-organized criticality). Finally, it also supports and extends the use of subthreshold membrane potential fluctuations as a carrier for some kinds of network state information.

Disclosures: J.K. Johnson: None. **N.C. Wright:** None. **R. Wessel:** None.

Poster

713. Visual Cortex Dynamic Properties

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Support: Wellcome Trust/DBT India Alliance

Tata Trusts Grant

DBT-IISc Partnership Programme

Title: Spatial spread of local field potential is band-pass in the primary visual cortex

Authors: *A. DUBEY, S. RAY;
Ctr. For Neurosci., Indian Inst. of Sci., Bangalore, India

Abstract: In recent years, local field potential (LFP), which is obtained by low-pass filtering the raw signal from a microelectrode inserted in the brain, has emerged as a promising candidate in understanding brain functions and in brain-machine interface (BMI) applications. However, there is no consensus about the cortical area which contributes to the LFP, known as its "spatial spread", with different studies reporting values between a few hundred micrometers[1] to several millimeters[2]. Further, dependency of spatial spread on frequency is also not well known, with previous modelling and theoretical studies predicting either "all-pass" (all frequencies spread equally) or "low-pass" (lower frequencies spread farther than higher frequencies) behavior.
We recorded brain signals from the primary visual cortex of two awake male rhesus monkeys using a 10x10 microarray. A small Gabor stimulus was flashed at different locations on the screen in a random order to estimate the visual spread for each recording site. The spatial spread

(measured in micrometers) was computed from the visual spread by using a model proposed by Xing and colleagues (2009)[1]. To estimate the spatial spread as a function of frequency, we first obtained the time-frequency spectrum of the signal using a signal processing technique called "Matching Pursuit", and subsequently estimated the visual spread at each frequency. Surprisingly, we found the LFP spread to be "band-pass", with frequencies in the high-gamma (60-150 Hz) range spreading significantly more than both lower (20-40 Hz) and higher (>250 Hz) frequencies. This was mirrored by an increase in phase coherence across neighboring sites in same frequency range, providing experimental evidence to a recent model[3] that reconciles different studies by showing that spatial spread depends on the extent of neuronal correlations. Our results are useful in understanding cortical architecture and communication, as well as in BMI applications.

References 1. Xing, D., Yeh, C.-I. & Shapley, R. M. Spatial Spread of the Local Field Potential and its Laminar Variation in Visual Cortex. *J. Neurosci.* **29,** 11540-11549 (2009).
2. Kajikawa, Y. & Schroeder, C. E. How Local Is the Local Field Potential? *Neuron* **72,** 847-858 (2011).
3. Lindén, H. *et al.* Modeling the Spatial Reach of the LFP. *Neuron* **72,** 859-872 (2011).

Disclosures: A. Dubey: None. **S. Ray:** None.

Poster

713. Visual Cortex Dynamic Properties

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Title: Critical network dynamics determines the variability of neural activity

Authors: *Y. KARIMIPANAH, Z. MA, R. WESSEL;
Washington Univ. In St.Louis, University City, MO

Abstract: A rigorous understanding of brain dynamics and function requires a conceptual bridge between multiple levels of organization, including neural spiking and network-level population activity. Among the vast spectrum of spatial and temporal scales of brain activity, two experimentally accessible levels of brain organization are (i) the single-neuron spiking and (ii) the population activity of the network in which the neurons are embedded to various degrees.

Single-neuron spiking in cerebral cortex is characterized by statistical properties, such as irregular spiking and reduced variability during sensory stimulation. Population activity is characterized by complex spatiotemporal activity, including scale-free activity, which is predicted to occur for a network state near criticality. These observations at two adjacent levels of brain organization raise the question, to what extent the network state controls the variability of single-neuron spiking?
To explore impact of the network state on the statistics of neuronal spiking, we used a model of binary probabilistic integrate and fire neurons. We simulated the network activity for different states, for which we quantified spiking irregularity in terms of *coefficient of variation* (CV) of the inter-spike-intervals and the variability of spike trains in terms of average Fano factor (FF) over a sliding time window. In our computational model we show that two prevalent features of cortical single-neuron activity, irregular spiking (CV>1) and the decline of response variability at stimulus onset, could both arise as emergent properties of a recurrent network operating near criticality. Importantly, our work reveals that the relation between the irregularity of spiking and the number of input connections to a neuron, i.e., the in-degree, is maximized at criticality. This relation establishes a valuable conceptual link between criticality and the important field of network theory. Furthermore, as the observed decline in response variability is regarded as an essential mechanism to enhance response fidelity to stimuli, our discovery of its relation to network criticality offers a starting point toward unraveling the possible roles of critical dynamics in neural coding.
It will be interesting to see to what extent the presented findings will generate a paradigm shift in the study of criticality of neural systems, as our results also provide novel and robust measures to test the criticality hypothesis. Overall, our findings establish criticality as a unifying principle for the variability of single-neuron spiking and the collective behavior of recurrent circuits in cerebral cortex.

Disclosures: **Y. Karimipanah:** None. **Z. Ma:** None. **R. Wessel:** None.

Poster

713. Visual Cortex Dynamic Properties

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Topic: D.06. Vision

Support: NSFC Grant 31271169

Title: Computational model on spatial-temporal response of primary visual cortex to contour stimuli

Authors: *J. LI[1], Y. YAN[2], W. WANG[3], W. LI[2], S. WU[2], D. WANG[3];
[2]Natl. Key Lab. of Cognitive Neurosci. and Learning, [3]Sch. of Systems Sci., [1]Beijing Normal Univ., Beijing, China

Abstract: Experimental study showed that population activities of primary visual cortical neuron encode the information of contour. Here we applied a physiology plausible computational model to explore the spatial-temporal dynamics of these population activities. We model a patch of neurons in V1 including 10*10 orientation hypercolumns. Excitatory (75%) and inhibitory (25%) neurons are randomly arranged in the plane and their preferred orientation forms a spinwheel structure. The short-range connections are isotropic among nearby neurons and mediated by AMPA or GABA receptors depending on the type of presynaptic neuron. The long-range connections are orientation-specific and mediated by NMDA receptors. We employ Wilson-Cowan model to describe the dynamics of single neuron. We apply the first order equation to model the fast synapse mediated by AMPA and GABA receptors and the second-order equation to model the slow synapse mediated by NMDA receptors. Given an image, we obtain the signal strength by filtering the image using steerable filters and the signal strength determines the input to a neuron according to its preferred orientation and spatial location.
Our model demonstrates typical spatial-temporal population activity of V1 upon orientation and contour stimuli. Given a stimulus with a single long straight line, the population activity pattern is consistent with classic image observations. Given a contour formed by five colinear bars with random background, the activation of neurons whose receptive field is on the contour are enhanced while the activities in the other areas are suppressed comparing with given stimulus of random bars (Fig). Comparing with the spiking neuron models, our firing rate model enables us to simulate large-scale cortical circuit efficiently and investigate the spatial temporal dynamics of large-scale network.

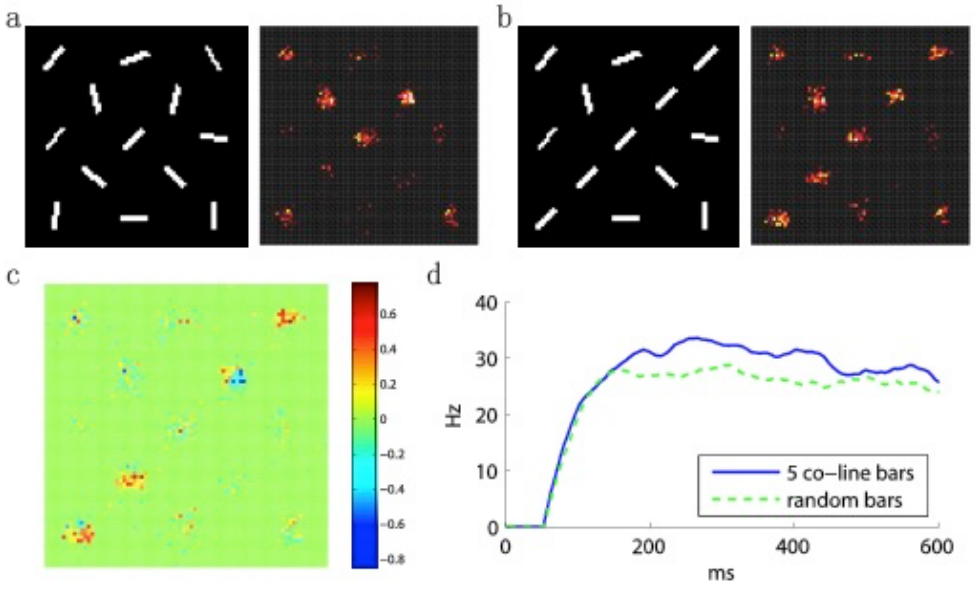

Fig: (a) Random background and the response. (b) Five colinear bars with random background

and the response. (c) Difference of responses in (a) and (b). (d) Average firing rate of the excitatory neurons in the center group.

Disclosures: **J. Li:** None. **Y. Yan:** None. **W. Wang:** None. **W. Li:** None. **S. Wu:** None. **D. Wang:** None.

Poster

713. Visual Cortex Dynamic Properties

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Topic: D.06. Vision

Support: Grants-in-Aid for Scientific Research 16K00220

Title: A role of lateral connections in the formation of orientation representation efficiently preserving visual information

Authors: ***M. MIYASHITA**[1], S. ISHIKAWA[2], J. HORIKAWA[2], S. TANAKA[3];
[1]Natl. Inst. of Technol., Numazu, Shizuoka, Japan; [2]Toyohashi Univ. of Technol., Toyohashi, Aichi, Japan; [3]The Univ. of Electro-Communications, Chyofu, Tokyo, Japan

Abstract: It has been believed for long time that preferred orientations are arranged tangentially in the mammalian primary visual cortex in an orderly manner, forming orientation maps. Recently, in the rodent visual cortex, however, preferred orientations have been found to be distributed almost randomly in a salt-and-pepper-like manner. These experimental findings raise a question of what functional roles cortical orientation representation plays in visual perception. Very recently, we have successfully reproduced not only orderly but also random orientation representations using our self-organization model of geniculo-cortical afferent inputs only by changing the value of the connection probability of lateral excitation p. The salt-and-pepper-like representation emerged with a large portion of unresponsive cells at $p < p_c$, whereas orderly maps without unresponsive cells appeared at $p > p_c$. In the present study, we examined to what extent visual images are reconstructed from activity patterns of cells in the model cortex that exhibits different types of orientation representations. The reconstruction was made so that the mean square error in activity patterns between a target image and a reconstructed one is minimized. We quantitatively evaluated the similarity of reconstructed images to target images. As a result, the larger the p value is, the higher the similarity is, as expected. In other words, the precision of image reconstruction is higher for denser lateral excitatory connections. Next we examined the similarity in case where simulations of self-organization had been conducted with lateral connections at $p < p_c$ or without lateral connections under the condition of the equal

number of responsive cells. The similarity was found to be conspicuously higher for the orientation representation that had been self-organized with lateral connections than for the representation without lateral connections. This suggests that cortical lateral connections are necessary for the development of the orientation representation that preserves visual information sufficiently to decode visual images, even if the representation is salt-and-pepper-like due to the sparseness of the connections.

Disclosures: **M. Miyashita:** None. **S. Ishikawa:** None. **J. Horikawa:** None. **S. Tanaka:** None.

Poster

713. Visual Cortex Dynamic Properties

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Title: Development of orientation selectivity in ferret V1: high cellular and columnar variability visualized through chronic 2-photon imaging

Authors: ***G. B. SMITH**[1], D. E. WHITNEY[1], B. HEIN[2], M. KASCHUBE[2], D. FITZPATRICK[1];
[1]Max Planck Florida Inst. For Neurosci., Jupiter, FL; [2]Frankfurt Inst. for Advanced Studies, Frankfurt am Main, Germany

Abstract: Selectivity for stimulus orientation is a fundamental property of primary visual cortex in primates and carnivores, where it is organized into a smoothly varying columnar map that emerges in an activity-dependent manner during early postnatal life. Numerous theoretical models have been proposed to explain the formation of orientation maps, and this key developmental process has been the subject of extensive experimental work. Despite these efforts, it has remained unclear how the modular structure of an orientation map forms over development and the degree to which individual neurons alter their preferences to produce a smooth map. In the ferret, intrinsic signal imaging shows that orientation maps first emerge around eye-opening and then exhibit a stable columnar pattern. However, this technique both lacks cellular resolution and requires extensive trial averaging, and therefore cannot distinguish between several potential explanations for the early absence of maps, including weak cortical responses, high trial-to-trial variability, and an intermixed ‘salt-and-pepper’ organization of

orientation preferences at the cellular level.
Here we address this limitation through the longitudinal imaging of GCaMP6s at both the columnar and cellular level in developing ferrets. We show that prior to eye-opening, gratings reliably evoke strong responses, which exhibit a highly modular structure with consistent domain size across both cortical locations and visual stimuli. Notably, the spatial location and pattern of domains activated by a single orientation varies greatly across trials, resulting in the absence of a clear orientation map. The high variability of orientation responses on the columnar scale is not a general feature of the immature cortex, as responses to uniform luminance steps are both selective and reliable. Longitudinal imaging shows that trial-averaged columnar orientation responses begin to show similarity to the future mature orientation map in the days immediately prior to eye-opening, despite response variability remaining high until several days following eye-opening. At the cellular level, two-photon imaging reveals that responses are spatially coherent prior to eye-opening, with no evidence of an intermixing of differently tuned populations. Similar to columnar responses, the responses of individual neurons also exhibited high trial-to-trial variability, which declined following eye-opening. Thus, our results suggest that the lack of strong orientation maps prior to eye-opening reflects highly variable responses in an immature cortex already exhibiting strong modular organization.

Disclosures: **G.B. Smith:** None. **D.E. Whitney:** None. **B. Hein:** None. **M. Kaschube:** None. **D. Fitzpatrick:** None.

Poster

713. Visual Cortex Dynamic Properties

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NSF CRCNS grant #1308174

Title: A model network with clustered connections reproduces the observed dynamics of correlated variability in visual cortex

Authors: *M. HOSEINI[1], N. WRIGHT[1], W. CLAWSON[2], W. SHEW[2], R. WESSEL[1]; [1]Physics, Washington Univ. In St. Louis, Saint Louis, MO; [2]Univ. of Arkansas, Fayetteville, AR

Abstract: The correlated variability (or “noise correlation”) of cortical responses to repeated presentations of the same stimulus originates from the interconnected nature of cortical circuits and impacts population coding. Two questions loom high in our quest to understand cortical signal processing: (1) What are the spatiotemporal dynamics of correlated variability? (2) What mechanisms mediate such dynamics of coordination?
To address the first question, we obtained simultaneous local field potential (LFP) recordings from a visual cortex in response to repeated visual stimulation. Specifically, we inserted a 96-channel microelectrode array into the visual cortex of the ex vivo turtle eye-attached whole-brain preparation and recorded the LFP responses to naturalistic movie clips projected onto the intact retina. We made four key observations with respect to the correlated variability among pairs of LFP recordings. First, correlated variability was small during ongoing activity, significantly increased at stimulus onset, and relaxed back to an intermediate level during continued visual stimulation. Second, correlated variability decreased with increasing inter-electrode distance. Third, correlated variability varied markedly among electrodes, but correlated variability of a pair during ongoing activity was related to the correlated variability during evoked activity. Fourth, LFP recordings featured intermittent oscillations; correlated variability among pairs of LFP recordings was strongly linked to the coherence of such oscillations.
To address the second question, focused on mechanisms, we investigated a model network of excitatory and inhibitory leaky integrate-and-fire (LIF) model neurons, including external inputs and synaptic depression with recovery. We mimicked two-dimensional space by arranging neurons on the surface of a sphere with a mix of mostly clustered and some long-range connections. We defined the simulated LFP as the sum of the synaptic currents within a group of adjacent neurons. Within this model framework, our simulations reproduced the experimentally observed temporal dynamics and the spatial dependence of correlated variability. In contrast, a random network with unstructured connections is not able to reproduce all experimentally observed features.
In conclusion, our work quantifies the spatiotemporal dynamics of correlated variability and emphasizes the role of structured connectivity in shaping the dynamics, thus building a bridge between cortical structure and computation.

Disclosures: **M. Hoseini:** None. **N. Wright:** None. **W. Clawson:** None. **W. Shew:** None. **R. Wessel:** None.

Poster

713. Visual Cortex Dynamic Properties

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Topic: D.06. Vision

Title: Spontaneous emergence of structured responses in a random neural network in-vitro

Authors: *M. SCHOTTDORF[1], J. VOGEL[1], H. SCHROBSDORFF[1], W. STÜHMER[2], F. WOLF[1];
[1]MPI DS, Goettingen, Germany; [2]MPI EM, Goettingen, Germany

Abstract: Neural networks with connections organized by probabilistic rules are conceptually powerful model systems. Among others, random neural networks have been shown to (1) generically exhibit computationally favorable properties for stimulus representation and information processing (e.g. Lukoševičius & H. Jaeger, 2009), (2) dynamically generate a state of sustained irregular spiking activity (van Vreeswijk & Sompolinsky, 1996) and (3) to account for visual cortical orientation selectivity (Ernst et al., 2001). What is left open in these theoretical studies is the question whether such ideas are viable in random networks of living cells.
We address this problem using a dissociated culture of rat cortical neurons. The neuronal connection patterns in such cultures are substantially less organized than neural circuits in the brain. We then drive these neurons optogenetically with spatially complex light patterns, generated by a holographic photostimulation system (Golan et al. 2009) and monitor neural responses optically with a redshifted genetically encoded calcium indicator (Dana et al. 2016 bioRxiv) together with ground truth data from a multielectrode array.
Stimulating the cell culture with moving gratings reveals a substantial degree of orientation tuning. We identify at least 33 out of 430 units from 16 cortical cultures as orientation selective, i.e. they show a circular variance < 0.9. This is highly significant with $p<0.001$**.** Notably, the orientation selectivity described here resembles to some extend cortical orientation selectivity.

Disclosures: **M. Schottdorf:** None. **J. Vogel:** None. **H. Schrobsdorff:** None. **W. Stühmer:** None. **F. Wolf:** None.

Poster

713. Visual Cortex Dynamic Properties

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Topic: D.06. Vision

Title: The computational tradeoff between dynamic range and stimulus discrimination in neural circuits near criticality.

Authors: *K. L. MCCLANAHAN, D. BOHLMAN, W. L. SHEW;
Physics, Univ. of Arkansas, Fayetteville, AR

Abstract: How the brain processes input from the senses depends crucially the collective interactions among neurons in the cerebral cortex and interactions among cortical regions. One important property of how a neural network encodes input is dynamic range, which quantifies the range of input intensities that lead to distinguishable responses. Previous experiments and computational models show that dynamic range is maximized when the interactions among neurons in the network are tuned to a special regime, called criticality. However, dynamic range is typically defined based on the average response to many repetitions of the same set of stimuli. This is unrealistic, because trial-to-trial variability can be high, especially at criticality, and the brain does not have the luxury of averaging over thousands of repetitions. A more principled approach, which accounts for trial-to-trial variability, is offered by signal detection theory. For a given pair of stimuli, the ability to correctly discriminate between the two stimuli depends on how the two distributions of responses overlap, and can be quantified as the area under the receiver operating characteristic (AUROC). Here, we study this problem using a network-level computational model of binary, probabilistic integrate-and-fire neurons. First, we consider a single network of neurons and show that the true distinguishability (AUROC) of stimuli is not optimal at criticality, but rather is highest in the subcritical regime. However, if we consider a second network of neurons that receives its input from the first network, the optimal regime for stimulus discrimination shifts back towards criticality. Considering the multi-network, hierarchical structure of real sensory systems (e.g. thalamus, primary sensory cortex, association areas, etc.), our results suggest that sensory input may be best distinguished when the system as a whole operates near criticality, but in the slightly subcritical regime. More generally, our results demonstrate a computational tradeoff: maximizing dynamic range comes at the cost of suboptimal stimulus discrimination.

Disclosures: **K.L. McClanahan:** None. **D. Bohlman:** None. **W.L. Shew:** None.

Poster

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Support: CIHR MOP 25825

CONICYT-BECAS CHILE

FESP-EOUM

Title: Pulvinar modulates the contrast response function of cortical neurons throughout the visual hierarchy.

Authors: *N. CORTES, B. O. SOUZA, J. LAI, U. KEYSAN, S. THOMAS, C. CASANOVA; Ecole d'optometrie, Univ. De Montreal, Montreal, QC, Canada

Abstract: While the properties of pulvinar cells and their connectivity with cortical visual areas have been described, their exact function are still unknown. Experimental and theoretical data suggest that projections from the pulvinar to the cortex modify the neural gain of the contrast response function (CRF) of cortical cells (Cortes and van Vreeswijk, 2012, 2015). Further, data from our group show that pulvinar inactivation can also shift the CRF of primary cortical neurons along the x-axis. How both gain and shift in the CRF can arise from the pulvinar? We explored this question by creating a network of sequential cortical levels attached to a pulvinar-like structure where each component is in the balanced state, to mimic the flow of neural activity through the visual system. We modeled the interactions between the visual cortices and the pulvinar with both reciprocal and non-reciprocal projections. While reciprocal connectivity was kept constant throughout the cortical chain, the synaptic strength of the non-reciprocal connections decreased as one moves from lower to higher cortical levels. We found that for early levels, reciprocal connections are mainly responsible for shifts in the CRF whereas local processing within the pulvinar together with non-reciprocal connections control the CRF gain across the cortical hierarchy. We compare these findings with experimental data on the CRF of V1 and 21a neurons of the cat prior to and after pulvinar inactivation. The proposed model suggests that both forms of CRF modulation could be managed sufficiently by cortico-pulvino-cortical connections, and does not require changes in cortico-cortical connection strength across the network.

Disclosures: N. Cortes: None. **B.O. Souza:** None. **J. Lai:** None. **U. Keysan:** None. **S. Thomas:** None. **C. Casanova:** None.

Poster

713. Visual Cortex Dynamic Properties

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Support: NIH Grant EY05253

Title: Effect of mean luminance on cortical responses to darks and lights

Authors: *R. MAZADE[1], J. JIN[1], C. PONS[1], H. SWADLOW[2], J. ALONSO[1];
[1]Biol. and Vision Sci., State Univ. of New York Col. of Optometry, New York, NY; [2]Dept. of Psychology, Univ. of Connecticut, Storrs, CT

Abstract: The cat primary visual cortex is organized in ON and OFF domains of neurons that show different preferences for light and dark stimuli. OFF domains generate stronger responses and have a more accurate representation of visual space than ON domains. It is currently unclear how this cortical OFF-dominance is affected by the different luminance levels of visual scenes. To better understand the relation between cortical OFF dominance and mean luminance, we performed horizontal penetrations through cat primary visual cortex with multielectrode arrays (32-electrode Neuronexus, 0.1 mm electrode spacing) and measured visual responses to dark and light stimuli with luminance spanning from 0.007 to 70 cd/m^2 (mean luminance was attenuated with neutral density filters placed in front of the eye). Our results demonstrate that, when driven by dark stimuli in light backgrounds, both OFF (dark on) and ON responses (dark off) double their strength as mean luminance increases from 0.007 to 0.7 cd/m^2. However, OFF responses remain consistently stronger than ON responses across the entire luminance range (average OFF vs. ON: 79 vs. 46 spk/sec, $p<0.001$, n=410 vs. 158 recording sites, Wilcoxon test). In turn, when driven by light stimuli in dark backgrounds, OFF responses (light off) are still stronger than ON responses (light on) but only at the higher luminance (ON vs. OFF: 61 vs. 80 spk/sec at 0.7-70 cd/m^2, n=310 vs. 395, $p<0.001$; 65 vs. 64 spk/sec at 0.007-0.7 cd/m^2, n=147 vs. 131, p=0.35; Wilcoxon tests). OFF responses were also stronger than ON responses for all stimulus conditions in mid-gray backgrounds except for lights at 7-70 cd/m^2 (light ON vs. light OFF: 51 vs. 45 spk/sec, n=212 vs. 127, p=0.009, Wilcoxon test). In addition to changes in response strength, increasing stimulus luminance caused a pronounced reduction in ON and OFF response latency, which could be accurately fit with a linear function in a semi-log scale. For each log-unit increase in luminance, ON response latency was reduced by 14.25 msec in dark backgrounds (R^2 = 0.98, n=4 luminance bins, p=0.007) and OFF response latency was reduced by 11.13 msec in light backgrounds (R^2 = 0.99, n=4 luminance bins, p=0.001). Surprisingly, increasing the mean luminance also made OFF receptive fields slightly larger (23% for a luminance increase from 0.07 to 70 cd/m^2 in light backgrounds, n=70 vs. 417, $p<0.001$, Wilcoxon test), an increase that reached half-saturation at 0.09 cd/m^2. These results demonstrate that mean luminance has pronounced effects on the response magnitude, latency and size of OFF cortical receptive fields and reveal a surprising dominance of the OFF pathway across a wide illumination range including low mesopic light.

Disclosures: R. Mazade: None. **J. Jin:** None. **C. Pons:** None. **H. Swadlow:** None. **J. Alonso:** None.

Poster

713. Visual Cortex Dynamic Properties

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Support: RPB

NSF

NIH

University of Utah research foundation

Title: Gamma oscillations as a marker of collinear horizontal and feedback connectivity in primate primary visual cortex

Authors: *M. BIJANZADEH[1], A. ANGELUCCI[2];
[1]Moran Eye Inst., Neurosci. program, Univ. of Utah, Salt Lake City, UT; [2]Moran Eye Inst., Salt Lake City, UT

Abstract: Orientation tuned surround modulation (SM) is a fundamental property of primary visual cortex (V1) neurons, thought to result from the interaction of horizontal (H) and feedback (FB) connections with local recurrent circuits (Angelucci et al. 2002; Shushruth et al. 2012). We previously proposed that both H and FB connections mediate the modulation from surround regions *near* the receptive field (RF), but only FB mediates *far* SM. H connections link neurons having similar orientation preference along an anisotropic axis in visual space collinear with the orientation preference of the connected neurons (Bosking et al. 1997). We have recently shown that FB connections may have a similar collinear arrangement (Federer et al., SFN 2015). Stimulation of the RF surround with isotropic gratings has been shown to increase the power in gamma oscillations (30-90 Hz) when the RF is simultaneously stimulated (Gieselmann & Thiele 2008). Gamma oscillations are thought to enhance information transmission across different brain regions (e.g. Fries 2009), and to reflect the activity of recurrent circuits (Borgers & Kopell 2008; Xing et al. 2012). If SM results from the interaction of visually anisotropic long-range connections with local recurrent circuits, then gamma oscillations induced by surround stimulation should reflect this anisotropy in connectivity. Here we have tested this hypothesis. Using linear electrode arrays in macaque V1, we recorded local field potential (LFP) responses to small (0.5-1.5°) iso-oriented grating patches flashed at varying distances from the RF center (0.5°-3.4°), in collinear or non-collinear configuration relative to the preferred orientation of neurons in the recorded V1 column. LFPs were filtered (1-100Hz) and their power spectrum was computed using Chronux toolbox (multitaper method, 8 Hz bandwidth, 3 tapers, Mitra & Bokil,

2008). The increase in gamma power was defined as the difference between the logarithm of the power during stimulus presentation (0-200ms) and prior to stimulus onset (-200-0ms).
We found that collinear surround grating patches in the absence of RF stimulation evoked higher gamma power than non-collinear patches in both superficial and deep V1 layers, and this effect decreased with distance from the RF. Moreover, collinear (but not non-collinear) patches in the near surround evoked greater increases in gamma power than far surround patches. These results suggest that collinearity is an important feature of the H and FB connectivity underlying near and far surround modulation, and support a role for recurrent networks in surround modulation.

Disclosures: M. Bijanzadeh: None. **A. Angelucci:** None.

Poster

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Title: Layer 6 corticogeniculate neurons in the awake visual cortex: Receptive field properties and effect of alert/non-alert brain states.

Authors: *C. R. STOELZEL[1], Y. I. BERESHPOLOVA[1], J.-M. ALONSO[2,1], H. A. SWADLOW[1,2];
[1]Dept. of Psychology, Univ. of Connecticut, Storrs, CT; [2]Dept. of Biol. Sci., SUNY-Optometry, New York, NY

Abstract: The lateral geniculate nucleus provides a powerful input to layer 4 (L4) and a less dense but significant input to layer 6 (L6) of the visual cortex. L6 corticogeniculate (CG) neurons provide a feedback projection to the LGN that is thought to shape receptive field (RF) properties and influence spike timing and response gain of thalamic neurons. Here, we examine the RF properties of antidromically identified CG neurons of primary visual cortex of awake rabbits, in both alert and non-alert states, and we compare these findings with recently studied L4 simple cells (Zhuang et al., 2013; 2014). We found that ~ 40% of CG neurons did not respond to visual stimulation in either brain state, and these unresponsive cells had significantly longer corticothalamic axonal conduction times (30.8 ± 1.9 ms) than visually responsive CG neurons (19.0 ± 1.7 ms). All visually responsive CG neurons (n = 42) had simple RFs with either a single

ON or OFF domain, or spatially separate ON and OFF domains, and all but one (97.6%) had an F1/F0 ratio > 1. L4 simple cells and visually responsive CG neurons had similar (low) spontaneous firing rates (CG = 0.45 ± 0.09 spk/s L4 simple = 0.55 ± 0.08 spk/s), visually evoked response rates (F1, CG = 11.9 ± 1.3 spk/s, L4 simple = 15.2 ± 1.5 spk/s) and response linearity (F1/F0, CG = 1.42 ± 0.04, L4 simple = 1.53 ± 0.03). However, L6 CG neurons: 1) preferred lower temporal frequencies (CG = 3.0 ± 0.2 Hz, L4 simple = 5.8 ± 0.9 Hz), 2) had wider RFs (CG = 4.1 ± 0.3°, L4 simple = 3.2 ± 0.1°), 3) had similar preferred spatial frequencies (CG = 0.21 ± 0.02 cpd, L4 simple = 0.20 ± 0.03 cpd), 4) but had a wider SF bandwidth (CG = 1.38 ± 0.16 oct, L4 simple = 0.68 ± 0.07 oct), 5) were less directionally selective (DSI, CG = 0.42 ± 0.05, L4 simple = 0.68 ± 0.03), 6) had longer latencies to visual stimulation (CG = 55.6 ± 2.1 ms,L4 simple = 37.5 ± 2.8 ms), and 7) responded in a more sustained manner to a stationary stimulus. Moreover, the effects of alertness were considerably stronger on layer 6 CG neurons than on layer 4 simple cells. For both cell classes, alertness significantly reduced response variability (Fano factor), enhanced visually evoked responses (F1) to stimuli moving in the preferred direction while also suppressing responses to stimuli moving orthogonal to the preferred direction. However, state effects on CG neurons were greater. Using a population coding model for orientation detection (Zhuang et al., 2014; Hei et al., 2014), we show that these effects of alertness on CG neurons --enhanced reliability, higher gain, and increased suppression to orthogonal orientations-could increase the speed of cortical feature detection, and do so to a much greater degree (~ 2-fold) than was seen for layer 4 simple cells.

Disclosures: C.R. Stoelzel: None. **Y.I. Bereshpolova:** None. **J. Alonso:** None. **H.A. Swadlow:** None.

Poster

713. Visual Cortex Dynamic Properties

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Topic: D.06. Vision

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Title: Fast spike interneurons in layer 6 of rabbit visual cortex: Comparison with layer 4 and effects of alert/non-alert brain states

Authors: *Y. I. BERESHPOLOVA[1], C. R. STOELZEL[1], J.-M. ALONSO[1,2], H. A. SWADLOW[1,2];

[1]Dept. of Psychology, Univ. of Connecticut, Storrs, CT; [2]Dept. of Biol. Sci., SUNY-Optometry, New York, NY

Abstract: Thalamocortical axons from the lateral geniculate nucleus (LGN) project largely to layer 4 (L4) of primary visual cortex (V1), but also send a significant projection into layer 6 (L6). Here we examine the visual response properties, geniculocortical connectivity, and effects of brain state (awake alert vs. non-alert) of putative fast-spike inhibitory interneurons (suspected inhibitory interneurons, SINs) in L6 of V1, and we compare these findings with those previously obtained (Zhuang et al., 2013; 2014) from SINs of L4. SINs of L6 had similar latencies to electrical and visual stimulation as L4 SINs, and cross-correlation analysis between spike trains of LGN neurons and retinotopically aligned L6 SINs confirmed monosynaptic connectivity. In comparison to L4 SINs, L6 SINs: 1) were less linear (F1/F0 ratios, L6 vs L4: 0.2 ± 0.04 vs 0.53 ± 0.04), 2) had similarly high spontaneous firing rates (L6 vs L4: 17.76 ± 2.03 spk/s vs. 21.96 ± 2.18 spk/s), but significantly lower modulated response amplitudes (F1, L6 vs L4: 10.09 ± 1.87 spk/s vs 32.97 ± 3.73 spk/s), 3) were less sensitive to contrast (C-50 values, L6 vs L4: 24.31 ± 2.41 % vs 10.83 ± 1.94 %), and 4) were far less reliable (Fano Factor, L6 vs L4: 2.03 ± 0.35 vs 1.33 ± 0.08). Receptive field tuning properties for orientation, spatial frequency and temporal frequency did not significantly differ between SINs of L4 and L6. Additionally, the alert state greatly enhanced response reliability and the response amplitude of visually evoked responses during optimal stimulation. In contrast to the diversity of state-dependent effects previously reported for L4 SINs (Zhuang et al., 2014), the effect of state on L6 SINs was stronger, and considerably more homogeneous, demonstrating an enhanced sensitivity to state.

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Poster

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Title: Optogenetic inactivation of V2 feedback to primate V1 affects contrast gain, receptive field size and near surround suppression

Authors: *L. NURMINEN, S. MERLIN, M. BIJANZADEH, F. FEDERER, A. ANGELUCCI; Moran Eye Ctr., Univ. of Utah, Salt Lake City, UT

Abstract: Inter-areal feedback (FB) connections are a prominent feature of the cortex whose role remains hypothetical, due to the difficulty of inactivating FB selectively and rapidly without silencing an entire cortical area. Neurons in primate area V1 receive FB inputs from areas V2, V3 and MT. V2 FB inputs encompass the receptive field (RF) and *near* RF surround of V1 cells, while V3 and MT FB inputs extend to the RF *far* surround (Angelucci et al. 2002). Here, we investigated the impact of optogenetically inactivating V2-to-V1 FB terminals on V1 responses . The inhibitory opsin ArchT was expressed in axon terminals of V2 FB cells by injecting AAV9-CaMKII.Cre and AAV9.CAG.Flex.Arch into V2 of 3 marmosets. After 4-6 wks, linear array recordings (24-channels) were made in V1 of anesthetized animals. Optogenetic silencing of FB terminals in V1 was performed by surface photostimulation with a green (532nm) laser coupled to a 400μm diameter optical fiber or collimator, producing a ~2mm diameter light spot. V2 was shielded from light. For each penetration (n=8), we measured size tuning and contrast response functions with and without FB inactivation. Photostimulation intensity was gradually increased while measuring V1 neurons' size tuning curves using drifting high contrast sinusoidal gratings of increasing size optimized for the recorded cells. The lowest light intensity evoking response changes was chosen for the reminder of the experiment. Each stimulus was presented twice in succession for 500ms (ISI 750ms) with photostimulation (500ms) randomized between the first and second presentation.

In a subset of V1 units modulated by light, V2 FB inactivation caused a shift in the peak of the size tuning curve towards larger grating diameters, and increased spike-rate at the shifted peak, thus effectively increasing RF and surround sizes, and reducing *near* surround suppression. Both RF and RF-surround sizes (grating diameter at peak summation and at plateau, respectively) approximately doubled after FB inactivation. In contrast, the size and strength of *far* surround suppression, which is likely mediated by FB from V3 and MT, were unaffected by V2 FB inactivation. V2 FB inactivation also affected the contrast response of V1 cells by reducing spike-rate at all contrasts, and increasing contrast gain.

We conclude that V2 FB increases near surround suppression and “shrinks” RF size in V1 cells via a contrast gain-dependent mechanism. These results are consistent with a model of surround suppression (Schwabe et al. 2006) in which reduced FB excitatory inputs to V1 RFs allows V1 cells to sum inputs over larger visual field regions, until a threshold for inhibition is reached.

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Poster

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Title: Visual dominance for darks increases with low light and optical blur

Authors: *C. PONS, R. MAZADE, J. JIN, M. DUL, Q. ZAIDI, J.-M. ALONSO;
Biol. Sci., State Univ. of New York, New York, NY

Abstract: We have previously shown that differences in the luminance response function of ON and OFF visual-pathways can explain asymmetries in processing dark versus light features in visual scenes. We now measure dark/light asymmetries in human vision across two orders of magnitude of background luminance by using neutral density filters (0.0136 to 136 cd/m^2), and confirm that differences in ON/OFF luminance responses explain multiple dark/light asymmetries in perception. First, we demonstrate that humans see high spatial frequencies better with full-contrast gratings of dark bars than light bars (14.3% difference in correct responses at 16 cpd, $p<0.001$, Wilcoxon tests here and below), but the difference disappears under low luminance, where high spatial frequencies are no longer visible. Second, we show that, although low light eliminates dark/light asymmetries in grating acuity, it enhances the asymmetries for visual salience of dark/light spots presented in noisy backgrounds (dark-light difference in correct responses of 11.29% increases to 22.01% in low luminance, $p<0.001$). We also show that dark/light asymmetries are enhanced by optical blur (7.95% at focus vs. 18.79% with -5 diopters and 24.68% with +5 diopters, $p<0.001$). Third, we demonstrate that, although visual resolution is higher for gratings of dark bars, full-contrast light dots on dark backgrounds are detected better than dark dots on light backgrounds (-36% dark-light difference in correct responses for dots of 1 monitor pixel $p<0.001$), a difference that disappears or reverses as background brightness increases. Finally, we use a computational model to demonstrate that ON luminance-response saturation explains all the asymmetries tested. The model assumes that ON luminance saturation occurs under different luminance conditions, an assumption that was confirmed with multielectrode recordings in cat visual cortex. In these recordings, reducing luminance from 35 to 0.35 cd/m^2 shifted the luminance that generated 50% of maximum response to light spots (L50) by two orders of magnitude, while keeping it below the middle luminance range available (average L50 in high vs. low luminance: 18.49% vs. 29.29% of maximum luminance, $p=0.003$). Reducing luminance also caused a pronounced increase in the exponent of the luminance response function (1.06 vs. 2.41, $p<0.001$), allowing a more precise sampling of the narrower luminance range. These results support the notion that dark/light asymmetries in vision are a

consequence of ON luminance response-saturation and suggest a possible explanation for the association between low-light/optical-blur and myopia: an under-stimulation of the ON-pathway.

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Poster

713. Visual Cortex Dynamic Properties

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Title: Structure + dynamics lead to function in a new computational model of macaque primary visual cortex

Authors: L. CHARIKER[1], *R. M. SHAPLEY[2], L. YOUNG[1];
[1]Courant Inst., New York Univ., New York, NY; [2]Ctr. for Neural Sci., New York, NY

Abstract: Our overall aim is the development of a coherent dynamical theory that explains cortical function as a direct consequence of known circuitry and neuronal interactions. To this end we constructed a new computational model of layer 4Cα in macaque V1 cortex, modeled as a large network of integrate-and-fire neurons. More than in previous models, we have tried to incorporate realistic neuroanatomy, following closely data on cell density, fraction of Excitatory (E) and Inhibitory (I) cells, connectivity probabilities as well as the fact that connections are isotropic and non-specific. One important new feature of the model was that LGN input to layer 4Cα was very sparse, consistent with what is known about the LGN projection to V1 (Silveira, Perry 1991; Angelucci, Sainsbury 2006). Feedback from layer 6 is also incorporated. Specific modeling goals were: (1) to investigate interrelations between structure and dynamics, and the resulting functions of V1; (2) to show how it is possible that sparse LGN input can lead to V1's visual capabilities; (3) to replicate the known diversity of visual properties across V1; (4) to assess the magnitude of recurrent or feedback excitation compared to feedforward. Goals (1)-(4) were achieved through analysis of model regimes that replicate simultaneously many experimental datasets. We found: (re goals 1, 2) the model generates Orientation-Selective (OS) cells through a complex, nonlinear, dynamic computation involving multiple factors, including strong, recurrent, OS input from neighboring E-cells, and recurrent inhibition that is non-selective for orientation; (3) model neurons show great diversity in many dimensions: firing rates and firing patterns, synchronization with the population gamma rhythm, orientation and spatial

selectivity, and modulation ratio; (4) no more than 15% of the E- input to an E-cell in the model comes from LGN; the rest comes from cortex through lateral interactions with other 4Cα neurons and from feedback via layer 6. Furthermore, like the real cortex, the model generates partially synchronized firing in the gamma band (30-90 Hz) when driven by drifting gratings. The model's temporal synchrony is stimulus dependent: there is very little synchronized firing in background, or at low contrast, but strong synchrony when the stimulus is high contrast, again like the real cortex (Henrie and Shapley 2005). The model's architecture and its dynamical interactions both play key roles in determining its background activity and responses to visual driving. As our model is realistic, we propose that a similar relationship holds in the real cortex.

Disclosures: L. Chariker: None. **R.M. Shapley:** None. **L. Young:** None.

Poster

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Support: NIH NEI

CIHR Postdoctoral Fellowship

Title: Feature-specific organization of cortico-cortical feedback connections in mouse visual cortex

Authors: *C. HUH, C. BENNETT, R. M. VEGA, J. P. PEACH, S. HESTRIN;
Dept. of Comparative Med., Stanford Univ., Stanford, CA

Abstract: The visual system consists of lower and higher areas that are reciprocally connected. Although much is known of the role of feedforward pathways in shaping the receptive field properties of neurons, relatively little is known about the role of feedback pathways in visual processing. Nine cortical areas that receive retinotopically-organized input from V1 have been identified in the mouse (Wang and Burkhalter, 2007). In particular, superficial neurons in two areas, AL (anterolateral) and PM (posteromedial), have been shown to possess distinct visual properties in terms of spatial and temporal frequency tuning (Andermann *et al.*, 2011; Marshel *et al.*, 2011). AL neurons respond best to visual stimuli with relatively low spatial and high temporal frequencies, while PM neurons prefer those with relatively high spatial and low temporal frequencies. Both of these areas send significant feedback to V1 (Wang *et al.*, 2011; 2012). It is not yet clear whether feedback connections from these areas convey feature-specific

information to V1 and what functional impact they have on receptive field properties of V1 neurons. To investigate these questions, we employed retrograde viral technology, macroscopic imaging of cortical areas sending feedback to V1, *in vivo* optogenetics and single-unit recordings in awake mice. We used channelrhodopsin-2-assisted targeted recordings to identify AL-to-V1 and PM-to-V1 feedback neurons. Our preliminary results indicate that AL and PM neurons providing feedback to V1 exhibit distinct visual stimulus preferences, similar to previously reported characteristics of superficial neurons in these areas. Using optogenetic silencing of feedback neurons, we found that the effects of feedback on V1 neurons exhibit like-to-like functional impact; V1 neurons whose visual properties are similar to the feedback area being silenced showed the greatest modulation in visual responses. For example, silencing PM feedback suppressed visual responses primarily in V1 neurons tuned to high spatial frequencies. We are currently investigating whether feedback effects on V1 neurons are modulated by the behavioural state of the animal, as indicated by spontaneous running. This study will reveal new insights into the functional organization of feedback connections in the brain and contribute to a greater understanding of the role of top-down pathways in vision.

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Poster

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Title: Somatostatin neurons drive long-range gamma band synchronization in the visual cortex

Authors: ***J. VEIT**[1], R. HAKIM[1], M. P. JADI[2], T. J. SEJNOWSKI[2], H. ADESNIK[1]; [1]UC Berkeley, Berkeley, CA; [2]Salk Inst., La Jolla, CA

Abstract: Rhythmic activity is a ubiquitous feature of brain circuits, and has been linked to many aspects of sensory and cognitive function. Gamma band rhythms, in particular, may synchronize distributed cell assemblies to facilitate information transfer within and across brain areas, yet their underlying mechanisms and functional consequences for neural computation

remain hotly debated. Most circuit models pose that soma-targeting parvalbumin (PV) positive GABAergic interneurons help mediate gamma rhythms through somatic inhibition. In contrast, using cell-type specific manipulations we show that dendrite-targeting somatostatin (SOM) interneurons are critical for visually induced cortical gamma rhythms in awake, behaving animals. Both a computational model based only on prior measurements of connectivity, and a brain slice model using optogenetic activation of horizontal cortical circuits, recapitulated gamma rhythms that depend critically on somatostatin interneurons. In vivo, SOM-mediated oscillations synchronize remote ensembles that process stimuli with common sensory features, implicating them in context-dependent visual perception. Taken together, these data establish a new mechanism for synchronizing distributed cell assemblies. By operating through dendritic rather than somatic inhibition, SOM-mediated oscillations may expand the computational power of gamma rhythms for optimizing the synthesis and storage of sensory perceptions.

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Poster

713. Visual Cortex Dynamic Properties

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Title: Contextual modulation and the cortical hierarchy: inactivating feedback from V2/V3 reveals multiple mechanisms of surround modulation in V1

Authors: *A. TROTT[1], S. G. LOMBER[2], R. T. BORN[1];
[1]Neurobio., Harvard Med. Sch., Boston, MA; [2]Dept. of Physiol. and Pharmacol., Brain and Mind Institute, Univ. of Western Ontario, London, ON, Canada

Abstract: One of the defining features of cortex is its hierarchical organization, defined by the feedforward and feedback projections that connect distinct cortical areas. Compared to feedforward processing, the functions carried out through cortico-cortical feedback are poorly understood, despite the anatomical abundance of these projections. Previous work from our lab established that feedback contributes to size tuning in V1, a canonical form of surround suppression (Nassi et al. 2013, 2014), and we sought to better understanding this contribution in

order to gain deeper insight into the computational role of feedback. The effects of the stimulus surround on V1 responses to natural stimuli have given rise to two prominent theoretical perspectives on surround modulation: that surround normalization is flexibly and optimally gated to promote the efficient representation of natural images (Coen-Cagli et al. 2015); and, that surround suppression effectively produces input-gain control (Lochmann et al. 2012). These mechanisms are expected to produce distinctive patterns of surround modulation, which we can disentangle through the careful design of parametric stimuli, including center 'plaids' with surround gratings (Trott and Born 2015). Here, we examined the effects of reversibly inactivating feedback from areas V2/V3 to V1 in awake macaque monkeys while recording responses to these parametric stimuli. Our preliminary results suggest that input-gain control remains intact during feedback inactivation, while divisive normalization is affected. We tentatively conclude that multiple underlying surround mechanisms contribute to contextual modulation in V1, potentially consistent with multiple theoretical frameworks, and that feedback contributes to surround mechanisms involved in flexible normalization.

Disclosures: **A. Trott:** None. **S.G. Lomber:** None. **R.T. Born:** None.

Poster

713. Visual Cortex Dynamic Properties

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Topic: D.06. Vision

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Title: Modelling transient dynamics and attentional modulation in MT cells in a structurally simple model

Authors: L. BOHNENKAMP, D. WEGENER, *U. A. ERNST;
Univ. Bremen, Bremen, Germany

Abstract: In the primate brain, area MT is largely responsible for the processing and perception of motion in visual scenes. MT neurons are tuned for the direction and the speed of moving stimuli and they respond with pronounced firing rate transients upon a change in visual stimulation. These transients increase the neurons' sensitivity and they are directly related to behavioural performances. Therefore, to gain a better understanding of the processing of dynamic visual scenes it is important to understand the neural processes that shape the transients in MT responses. We present a dynamical model of MT cells that takes the cells' speed and direction tuning into account and that is able to reproduce their experimentally observed

transients. In its simplest version, the model consists of only two coupled differential equations, representing an excitatory and an inhibitory population that make up a single direction column. Both populations receive the same external input and additionally the inhibitory population provides divisive inhibition to the excitatory one. Using a simple optimization procedure, we fit the model to find close to perfect fits for the sustained and transient phases in MT single cell responses. Based on the fit for each cell, the model reproduces and predicts neuronal responses to arbitrary accelerations and decelerations of a moving stimulus starting from both low and high base speeds, including experimentally observed deviations of the actual transient as compared to the expected firing rate change as deduced solely from a neuron's speed tuning profile. We also investigated the possible effects of spatial and feature-directed attention in our model and reproduced psychophysical reaction time distributions under different attentional conditions. In particular, we found increasing relative firing rate differences during the transients the stronger the attentional modulation was prior to a stimulus change. This effect reversed in the sustained phase of the neurons' response, where relative activation decreased with increasing attentional modulation. Our finding provides a mechanistic explanation for the shaping and attentional modulation of neuronal response transients, and underlines the importance of this dynamical mechanism for change detection.

Disclosures: **L. Bohnenkamp:** None. **D. Wegener:** None. **U.A. Ernst:** None.

Poster

714. Balance, Posture, and Orientation

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Topic: D.07. Vestibular System

Support: AFOSR FA9550-12-1-0395

Title: Learning dynamic balancing with and without gravitation dependent cues

Authors: ***V. P. VIMAL**, P. DIZIO, J. R. LACKNER;
Brandeis Univ., Waltham, MA

Abstract: Our objective was to compare learning in a dynamic self-balancing task when gravitational cues relevant to position were present or absent. Subjects sat blindfolded in a device that was programmed to behave as an inverted pendulum about the body roll axis, which could be either upright or supine. They used a joystick to align themselves to the direction of balance. Each subject was tested over twenty 100 second trials on two consecutive days in one of four groups (n=10/group). Group 1 had upright balancing on both days; Group 2 had supine balancing

on both days; Group 3 had upright balancing on the first day and supine balancing on the second day, and Group 4 had the opposite order. To quantify learning we used metrics derived from phase portraits and stabilogram diffusion function analysis of roll plane motion and joystick deflections. Subjects tested upright on Day 1 showed significant improvement, learning to reduce the standard deviation of angular position, the frequency of crashes (angular deviations from the direction of balance greater than $\pm 60^0$), the magnitude of joystick deflections, destabilizing joystick deflections, the overall energy of the system; and learning to increase the intermittency of joystick commands and the time interval between short term persistent motion and the long term anti-persistent motion. Subjects tested supine on Day 1 showed minimal improvement, learning to reduce only the frequency of crashes and destabilizing joystick deflections. While a subset of supine subjects showed improvements on Day 1, all supine subjects showed positional drift and most reported uncertainty about their position. Subjects tested on Day 2 in the same condition as Day 1 (Groups 1 and 2) showed retention of their Day 1 learning. Prior exposure to the upright condition did not enhance learning of supine balancing (Group 3), however prior exposure to the supine condition did accelerate learning of upright balancing (Group 4). Our results indicate that in the absence of gravitation dependent otolith and somatosensory cues about dynamic body orientation the ability to balance is significantly impaired.

Disclosures: V.P. Vimal: None. **P. DiZio:** None. **J.R. Lackner:** None.

Poster

714. Balance, Posture, and Orientation

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Topic: D.07. Vestibular System

Support: EU-Project 611452 (ABBI)

Title: Successful balance training improves memory and spatial cognition

Authors: *A.-K. ROGGE[1], B. ROEDER[1], A. ZECH[3], V. NAGEL[2], K. HOLLANDER[2], K.-M. BRAUMANN[2], K. HOETTING[1];
[1]Biol. Psychology and Neuropsychology, [2]Human Movement Sci., Univ. of Hamburg, Hamburg, Germany; [3]Human Movement Sci., Univ. of Jena, Jena, Germany

Abstract: Physical exercise has been shown to improve memory and spatial cognition and to induce structural changes in the hippocampus. In humans, these effects have mostly been studied after aerobic exercise. However, it remains unknown whether an increase in cardiovascular fitness is a prerequisite for the beneficial effects of physical exercise on cognition. Some findings

suggest that the vestibular system might mediate this link, as (a) it codes self-motion which is inherent in any physical exercise; (b) it provides direct connections to the hippocampus; and (c) vestibular lesions have been shown to result in memory and spatial cognition deficits. Physical exercises with high demands on balance skills stimulate the vestibular senses while leaving the cardiovascular fitness unaffected. Therefore, we examined the effects of a controlled balance training intervention on memory and spatial cognition. Healthy adults (N=40, 19-64 years) were randomly assigned to a diversified balance training and to a muscle relaxation control group. Both groups trained twice a week for three months. Before and after training, participants were tested with a dynamic balance task on a moveable platform and a stepwise ergospirometry to measure cardiovascular fitness (maximum oxygen uptake, VO_2peak). Memory was assessed by an auditory verbal paired-associate learning task. Spatial cognition was assessed by small scale mental rotation as well as orienting and perspective taking tasks, consolidated into one composite spatial score. The balance training group showed significantly higher scores at post-test than at pre-test for the balance parameter, whereas the control group did not improve across sessions. The increase in balance skills in the balance training group was accompanied by an improvement in memory and spatial performance: Whereas there were no differences between groups prior to training, the balance training group outperformed the control group in the paired-associate learning and in the spatial score after training. There was no change in VO_2peak over time in either group. The present results suggest that successful balance training improves memory and spatial cognition. These findings suggest that a stimulation of the vestibular system might contribute to the beneficial effects of physical exercise on neurocognitive functioning, and that an increase of cardiovascular fitness is not a prerequisite for these effects.

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Poster

714. Balance, Posture, and Orientation

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DLR

AMC

Title: Visual reinforcement of illusory rotation during centrifugation: A novel habituation strategy to motion sickness

Authors: ***G. I. BERTOLINI**[1], J. E. BOS[2], D. BRON[3], T. FRETT[4], E. GROEN[2], R. HEMMERSBACH[4], F. L. WHETS[5];
[1]Dept. of Neurology, Univ. Hosp. Zurich, Zurich, Switzerland; [2]TNO, Soesterberg, Netherlands; [3]Aeromedical Inst., Duebendorf, Switzerland; [4]Inst. of Aerospace Med., German Aerospace Ctr., Cologne, Germany; [5]Antwerp Univ. Res. centre for Equilibrium and Aerospace, Univ. of Antwerp, Antwerp, Belgium

Abstract: Background: Artificial gravity through centrifugation is currently the only countermeasure providing an "Earth-like" solution to weightless health hazards. Every head movement during centrifugation, however, may cause motion sickness due to conflicts between the perceived direction of gravity and the illusory rotations caused by the vestibular activation (Cross-coupling stimulus). Existing habituation protocols are based on repetition of cross-coupling stimuli. Although they successfully abate motion sickness, they also reduce overall responses to rotations. Our aim is to develop a novel habituation strategy to disentangle gravity and rotation perception, reducing motion sickness but retaining response to rotation. Methods: We tested 19 healthy subjects on the ESA Short Arm Human Centrifuge at DLR, Cologne. The control group (CG: 9 subjects, 3 female) performed a normal habituation protocol consisting of repetitive 30° clockwise head rolls during centrifugation at 100°/s (1 g at feet). The test group (TG: 10 subjects, 5 female) performed an identical protocol, with the addition of visual stimuli triggered by head movements providing an illusion of rotation (optokinetic stimuli provided through the Oculus Rift) assumed to reinforce the vestibular activation. Motion sickness was measured using a 1-20 scale, while an eye tracker embedded in the Oculus Rift recorded the vestibulo-ocular reflex (VOR). Habituation measurements were obtained repeating the experiment after 24h. Results: 15 subjects (7 CG, 8 TG) were able to complete the experiment. No difference between groups was observed in the motion sickness score on day 1 or in the reduction of motion sickness from day 1 to day 2 (median [MAD] CG: -4 [2]; TG: -4 [1], p=0.78). The CG, however, had a significantly larger reduction of the VOR duration than the TG (CG: -4 [1] s; TG: -1 [2] s, p=0.05). Conclusions: Subjects habituate to the cross-coupling stimuli during centrifugation even if illusory self-rotation induced by head tilts is sustained by visually induced perception of self-rotation. Visually reinforced habituation may however induce less reduction of oculomotor response to rotation than classical habituation and may therefore better preserve vestibular reflexive responses.

Disclosures: **G.I. Bertolini:** None. **J.E. Bos:** None. **D. Bron:** None. **T. Frett:** None. **E. Groen:** None. **R. Hemmersbach:** None. **F.L. Whets:** None.

Poster

714. Balance, Posture, and Orientation

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 714.04/JJ6

Topic: D.07. Vestibular System

Support: FP7-ICT 611452

Title: The influence of roll tilt on perceptual verticality in children

Authors: *L. F. CUTURI, M. GORI;
U-Vip, Inst. Italiano Di Tecnologia, Genova, Italy

Abstract: Orientation of head and body in the roll plane is known to affect perception of verticality causing the so-called A-effect or E-effect, respectively biases towards or away from the tilt angle. These effects have been extensively studied in adults focusing on the readout of vestibular information and the sensory modality involved in the estimation of verticality. Although previous studies have shown that visual and tactile modalities do not optimally combine until adulthood and they contribute differently to the perception of stimulus orientation, less is known about the role of vestibular information in influencing such perceptual modalities during development. To fill this gap, we investigated how head and body roll tilt of 90° relative to gravity influences perception of verticality in children (5-13 years). We had participants lying on one side and asked them to evaluate stimulus orientation either haptically by touching a plastic bar or visually by observing a luminous bar. Participants had to report whether the stimulus was tilted more clockwise or counterclockwise compared to verticality. In order to control for differences given by age, we performed the same experiment in a group of adult participants. For all groups and conditions, we employed an adaptive procedure to select the stimulus orientation to be presented thus allowing us to quantify participants' biases and variability in the estimation of verticality. Our findings show different patterns of results depending on participants' age and involved sensory modality. Adults and older children tend to show E-effects in the tactile modality whereas the youngest group of participants (5-7 years) shows an opposite tendency. Although visual precision tends not to differ across age groups, these results suggest a stronger role of vision in perception of verticality in line with previous findings showing vision to overcome touch in processing stimulus orientation at the early stages of development. Regardless of age, all participants show A-effects in the visual condition with a tendency for the youngest participants (5-7 years) to show less pronounced biases. These results suggest an effect of age on the processing of vestibular information: idiothetic priors in young children seem to be less influential and their reliance on vestibular sensory information might be higher compared to adults who instead might rely more on prior experience (e.g. standing upright). Overall, these findings throw light on how the brain interprets vestibular sensory

information at different stages of development considering its role in influencing other sensory modalities as visual and tactile.

Disclosures: **L.F. Cuturi:** None. **M. Gori:** None.

Poster

714. Balance, Posture, and Orientation

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Support: NIH grant F31NS095491

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Title: Effects of chronic Purkinje cell silencing on central processing of vestibular self-motion signals

Authors: ***T. STAY**, J. LAURENS, D. E. ANGELAKI, R. V. SILLITOE;
Neurosci., Baylor Col. of Med., Houston, TX

Abstract: The vestibular otolith organs physically cannot distinguish between linear inertial acceleration and changes in head position relative to gravity. Previous research advocates that the two types of motion are separated centrally by combining otolith information with signals from the semicircular canals. Notably, neurons in the cerebellum and vestibular nucleus modulate their firing rates to represent linear acceleration or gravitational changes, with a broad range of selectivity. Since experimental evidence suggests that cerebellar circuitry is significantly involved in the creation of internal models, we hypothesized that processing in the cerebellar cortex is necessary for single neurons to differentiate linear translation and gravitational tilt. To test this hypothesis, we adapted motion test protocols previously used for macaques to similarly test a published conditional genetic mouse line, $L7^{Cre};Vgat^{flox/flox}$. Our lab developed this line to constitutively prevent cerebellar Purkinje cells from signaling to their targets through GABA. We recorded extracellular signals from Purkinje cells in awake mutant and littermate mice during combinations of translation and tilt. Our data first indicated that mice typically represent the two types of motion analogous to that reported for monkeys. Intriguingly, $L7^{Cre};Vgat^{flox/flox}$ mice also appear to have Purkinje cells which respond selectively to tilt or translation. This could be because feedback from Purkinje cell target areas into cerebellar cortex is not required for proper self-motion dissociation, or could also be reflecting a functional developmental

compensation. We are currently examining the vestibular circuit at other times and cell types to identify how Purkinje cell signaling shapes proper vestibular sensation.

Disclosures: **T. Stay:** None. **J. Laurens:** None. **D.E. Angelaki:** None. **R.V. Sillitoe:** None.

Poster

714. Balance, Posture, and Orientation

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Program#/Poster#: 714.06/JJ8

Topic: D.07. Vestibular System

Support: CIHR

CRC

Title: Second-order statistics of self-motion signals experienced by human subjects during everyday activities: implications for vestibular processing

Authors: ***M. J. CHACRON**[1], J. CARRIOT[1], M. JAMALI[1], K. E. CULLEN[2];
[2]Physiol., [1]McGill Univ., Montreal, QC, Canada

Abstract: Understanding the neural code remains a central problem in systems neuroscience. There is accumulating evidence that the brain's neural coding strategies are constrained by the statistics of the natural environment in which the organism lives. Thus, knowledge of natural stimulus statistics is essential towards understanding the neural code. Here we investigated the statistics of the time varying envelope (i.e. a second-order stimulus attribute that is related to variance) of rotational and translational self-motion signals experienced by human subjects during everyday activities. We found that second-order stimulus attributes can reach large values across all six-motion dimensions (~450 deg/s and ~4 G). Unlike results obtained in other sensory modalities, spectral power decreased slowly for low (< 2 Hz) and more sharply for high (>2 Hz) temporal frequencies and thus was not well-fit by a power law. We further found that this unique structure was not due to biomechanical filtering by the body. Instead, while the power spectra of signals resulting from passive self-motion were well-fit by a power law, those of signals resulting from active self-motion were not. Our findings have important consequences for understanding how natural second-order self-motion signals are processed by the vestibular system.

Disclosures: **M.J. Chacron:** None. **J. Carriot:** None. **M. Jamali:** None. **K.E. Cullen:** None.

Poster

714. Balance, Posture, and Orientation

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Topic: D.07. Vestibular System

Support: NIH Grant K23DC013552

Leon Levy Foundation

Fight for Sight Foundation

Title: Transcranial magnetic stimulation (TMS) of supramarginal gyrus tilts perception of upright without changing ocular torsion

Authors: *A. KHERADMAND[1], J. OTERO-MILLAN[2], A. WINNICK[2];
[1]Neurol., Eye Movement/Vestibular Lab., Baltimore, MD; [2]Neurol., Johns Hopkins Univ., Baltimore, MD

Abstract: Torsional position of the eyes or ocular torsion affects our perception of upright. Here we studied the functional role of the right temporal parietal junction (TPJ) in perception of upright by using transcranial magnetic stimulation (TMS). Perception of upright was measured by subjective visual vertical (SVV) during head tilt and ocular torsion was recorded simultaneously by video-oculography. The continuous theta burst stimulation (cTBS) over the posterior aspect of the supramarginal gyrus (SMGp) in 12 right-handed subjects tilted the SVV in the opposite direction of the head tilt without commensurate changes in ocular torsion. These findings suggest that the inhibitory effect of cTBS at the supramarginal gyrus alters perception of upright without changing torsional position of the eyes.

Disclosures: A. Kheradmand: None. **J. Otero-Millan:** None. **A. Winnick:** None.

Poster

714. Balance, Posture, and Orientation

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Topic: D.07. Vestibular System

Support: CIHR Grant

NIH Grant

Title: Head movements during locomotion in vestibular schwannoma patients: Decreased variability after unilateral vestibular lesion

Authors: *O. ZOBEIRI[1], C. ZHANG[1], S. KING[2], R. F. LEWIS[2], K. E. CULLEN[1];
[1]Dept. of Physiol., McGill Univ., Montreal, QC, Canada; [2]Dept. of Otolaryngology, Harvard Med. Sch., Boston, MA

Abstract: The vestibular system plays a key role in a wide range of functions from basic reflexes to high-level behavior. By detecting head motion and then generating the appropriate reflexes, the vestibular system is vital for maintaining balance and stabilizing gaze. In turn, it is well known that immediately following unilateral vestibular loss, patients experience impaired balance, postural, and gaze control. However, to date, much less is known about the effects of vestibular loss on voluntary behaviour. Here, to assess how vestibular loss alters voluntary behaviour, we analyzed locomotive behaviour in a group of patients with a diagnosis of vestibular schwannoma (VS) who had undergone a primary surgical resection of their tumor via suboccipital craniotomy and retrosigmoid approach with sectioning of the vestibular nerve. Head movements were recorded before the surgery, as well as 2 and 6 weeks after surgery using a micro-electromechanical systems (MEMS) module (Carriot et al., 2014), which combines three linear accelerometers (recording linear accelerations along the fore-aft, inter-aural, and vertical axes) and three gyroscopes (recording angular velocity about pitch, roll, and yaw). Patients were asked to complete the Functional Gait Assessment, and we focused our analysis on short, 15-30-second-long gait tasks including: normal walking, walking with eyes closed, and walking backwards on a level surface. We then compared pre-operative and post-operative data to determine if and how patient' movements were altered. We quantified gait speed, gait cycle asymmetry, and gait variability. Surprisingly, we found that locomotor speed actually increased six-weeks post-surgery as compared to pre-operative testing, and that movement asymmetry did not change. Furthermore, although previous studies using age-matched controls have found increased variability in gait, we found that this was not the case when using the pre-operative patient as their own control. Instead, movement variability was lower two weeks after the surgery than before the surgery. Specifically, the standard deviation of both linear acceleration and angular velocity in all dimensions (up-down, left-right, fore-aft, roll, pitch, and yaw) consistently decreased after surgery. Variability is often seen as the result of noise in the nervous system, however it has been shown that variability could contribute to motor learning. Taken together, our results suggest that variability is an important indicator of how patients are adapting to altered motion and the recovery process.

Disclosures: O. Zobeiri: None. **C. Zhang:** None. **S. King:** None. **R.F. Lewis:** None. **K.E. Cullen:** None.

Poster

714. Balance, Posture, and Orientation

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Program#/Poster#: 714.09/JJ11

Topic: D.07. Vestibular System

Support: CIHR

Title: Vestibular coding strategies for representing natural selfmotion. Differential integration of canal and otolith Inputs in the vertical and horizontal plane

Authors: *J. CARRIOT[1,2], I. MACKROUS[1], M. JAMALI[1], K. CULLEN[1];
[1]Physiol., McGill Univ., Montreal, QC, Canada; [2]Univ. of Western Ontario, London, ON, Canada

Abstract: During every day activities, self-motion is sensed by the vestibular system, which contributes to an impressive range of brain functions from the most automatic reflexes to spatial perception and motor coordination. It is generally assumed that sensory systems efficiently encode natural stimuli using coding strategies that are adapted to the statistics of the environment in which the organism lives. However, to date, sensory coding has been primarily studied using artificial stimuli. We recently characterized natural vestibular stimuli during everyday activities. One of the characteristic of these stimuli is their multidimensionality and the simultaneous activation of the canals and otolith organs. How these signals are integrated when the head motion is perform in the horizontal plane versus in the vertical plane remain unknown. We characterized the response of the vestibular only (VO) neurons receiving input from otolith and/or semicircular canal afferents. We recorded from central vestibular neurons in response to i) translation (otolith stimulation), ii) rotation in the horizontal plane (horizontal canal stimulation), iii) Tilt-translation (vertical canal stimulation), iv) multidimensional stimuli (in the horizontal and vertical plane), and v) while the monkey was generating comparable self-motion. Consistent with previous results, we found that the firing rate response to unidimensional stimuli was accurately estimated by a typical linear model, which could account for up to ~95% of the variance in the data. In contrast, during multidimensional motion, VO neurons sub-additively integrated the translational and rotational inputs rather than simply adding the otolith and canal afferents information. This sub-additivity was comparable in the vertical and horizontal plane ($p<.05$). Finally, we found that neuronal responses were significantly attenuated (~75%) during self-generated vs passively applied movements. Taken together, these findings provide insights into the underlying processing required for the integration of rotational and translational inputs in the vestibular system and are key for understanding the neuronal mechanisms required for accurate posture and motor control, as well as perceptual stability during everyday life.

Disclosures: J. Carriot: None. **I. Mackrous:** None. **M. Jamali:** None. **K. Cullen:** None.

Poster

714. Balance, Posture, and Orientation

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 714.10/JJ12

Topic: D.07. Vestibular System

Title: Ocular correlates of auditory vection

Authors: *D. GRENET[1,2], A. A. MIGLIACCIO[1,2], R. C. FITZPATRICK[1,2];
[1]Neurosci. Res. Australia, Randwick, Australia; [2]UNSW Australia, Sydney, Australia

Abstract: Sensations of self-motion, whether real or illusory, may be accompanied by reflexive eye-movements or changes in eye position. There are contradictory reports regarding the presence of these ocular correlates during auditory vection or illusory self-motion perception. Both ears (pinnae) from ten subjects were cast in silicone rubber. The silicone ears were mounted on a mannequin head and microphones were placed on the medial side of the silicone auditory canal. The ambient sounds of the laboratory and a variety of preprogramed sound stimuli created a soundscape that was acquired by these microphones, amplified and played to the subject through headphones as they sat in a dark booth within the laboratory to isolate them from other sound. Thus, the subject heard the soundscape as if he or she were at the position of the mannequin head with the sound filtered by their own pinnae. All subjects perceived the sounds as external to their head and could accurately localise their sources. We rotated the mannequin head in yaw at 2 constant speeds (20 and 50 degrees/s) and asked subjects to report their perceptions of self-rotation by clicking a button every time they had rotated 45 degrees. Left eye position was recorded using an EyeSeeCam video-oculography system (EyeSeeTech, Germany). Nine subjects reported feeling some self-motion, all as yaw rotations, in a total of 104/132 trials. Nystagmus was not observed, although eye deviations in the direction of perceived rotation resulted in significant ($p<0.05$) correlation between eye position and perceived rotational velocity in 40 % of the trials where subjects felt movement. These eye position deviations had amplitudes up to 20 degrees and were akin to the “shift of beating field” that often accompanies nystagmus evoked by vestibular or visual stimuli - a phenomenon shown to depend on the orientation strategy used by the subject, but never previously reported in the absence of nystagmus. An alternate explanation may be that perception of rotational motion in the absence of a dynamic vestibular stimulus is interpreted by the vestibular system as a static vestibular stimulus with an ocular response akin to the otolith-mediated head tilt response.
The presence of eye position deviations in the absence of nystagmus suggests that this eye movement is either a residual component of suppressed nystagmus or it is driven by a separate mechanism.

Disclosures: D. Grenet: None. **A.A. Migliaccio:** None. **R.C. Fitzpatrick:** None.

Poster

714. Balance, Posture, and Orientation

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Topic: D.07. Vestibular System

Support: NASA HRP-HHCE (Wood)

NASA NNX09AL14G/NNX12AM25G (Moore)

Title: The effect of 30-hr acute sleep deprivation on psychomotor vigilance and a Mars rover simulation

Authors: E. J. BELTRAN[1], S. T. MOORE[2], *S. J. WOOD[3];
[1]Wyle Science, Technol. and Engin. Group, Houston, TX; [2]Icahn Sch. of Med. at Mount Sinai, New York, NY; [3]Azusa Pacific Univ., Azusa, CA

Abstract: Increases in variability of docking misalignment have been observed in astronauts during a Mars rover simulation following long-duration spaceflight. The objective of this study was to examine the effect of 30-hr acute sleep deprivation on this simulation using ground control subjects to determine the influence of sleep loss independent of post-flight neurosensory adaptation. Nine subjects (39 ± 11 yr; 4F/5M) participated in three baseline laboratory sessions, a 30-hr sleep deprivation period, and a post-sleep deprivation laboratory session. The rover simulation consisted of a serial presentation of tasks that were self-paced to be completed as quickly and accurately as possible. The tasks consisted of (1) perspective-taking, using a map that defined a docking target, (2) navigation toward the target over simulated Martian terrain, and (3) docking a side hatch of the rover to a visually guided target. The simulator used a Stewart-type motion base (CKAS, Australia), a single-seat cabin with triple scene projection, and a joystick controller. Throughout the 30-hr sleep deprivation period, subjects reported subjective sleepiness via the Stanford Sleepiness Scale (SSS) and performed a 3-minute Psychomotor Vigilance Test (PVT) administered on an iPad (Joggle Research). The SSS and PVT were completed at 5-hr intervals during the first 10 hours, 2-hr intervals during the next 10 hours, and 1-hr intervals during the final 10 hours, with the last ones completed in the lab just prior to the rover simulation. There was a significant increase in SSS during the sleep deprivation period. Although mean SSS reported in the lab was slightly reduced from the last one reported at home, this 30-hr SSS was still significantly higher than that reported in the baseline sessions. The PVT mean reaction time (mRT) scores were significantly related to SSS, and were therefore used as the main outcome variable for the PVT. There was a significant increase in the mRT during the sleep deprivation period; however, the mRT at 30 hr in the lab was not different than the first mRT obtained at the beginning of the sleep deprivation period. There was also no significant change in any of the Mars rover simulation parameters between the last baseline session and the

post-acute sleep deprivation session. These results suggest that subjects had remained as vigilant during the lab session following the 30-hr sleep deprivation period as they were during the final baseline session, although they were subjectively more tired. We conclude it is important to control for behavioral alertness when testing for the neurosensory consequences of sleep loss.

Disclosures: E.J. Beltran: None. **S.T. Moore:** None. **S.J. Wood:** None.

Poster

714. Balance, Posture, and Orientation

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NIH grant DC013635 (FK)

Title: Sensory precision limits behavioral precision in a manual control task

Authors: M. J. F. ROSENBERG[1,2], R. GALVAN-GARZA[3], T. K. CLARK[4], D. P. SHERWOOD[3], L. R. YOUNG[3], *F. KARMALI[1];
[1]Otolaryngology, Jenks Vestibular Physiol Lab, Harvard Med. Sch., Boston, MA; [2]NASA Johnson Space Ctr., Houston, TX; [3]Man Vehicle Lab., MIT, Cambridge, MA; [4]Univ. of Colorado at Boulder, Boulder, CO

Abstract: Precise motion control is critical to human survival, whether running from prey on soft ground at night or piloting a spacecraft when landing on the moon. Sensorimotor responses and perception are inherently imprecise, and the sources of imprecision are not well known, nor are the functional implications of this imprecision. For example, there is disagreement about whether sensory imprecision dominates visual tracking, or if motor imprecision also contributes. Likewise, modeling has suggested that vestibular sensory imprecision contributes to postural instability. To more directly address these questions, we determined whether vestibular perceptual thresholds, which have substantial inter-subject variation, predict performance in a "vestibular" manual control task. In this task, subjects attempted to keep themselves upright using a control joystick to null out pseudo-random, head-centered roll tilt motion disturbances in the dark. Furthermore, we examined whether behavioral precision would change in an altered gravity environment. The "G level" was manipulated using rotation of a short-radius centrifuge, with roll tilt motion and the chair configured so that the manual control task occurred relative to centripetal accelerations of 0.5, 1.0, 1.3 G. Manual control performance was quantified by taking

the standard deviation of the chair position (i.e. worse nulling corresponded to less stability in chair position). Vestibular precision was quantified using direction recognition thresholds, the minimum movement that one can reliably distinguish as leftward vs. rightward. Subjects were roll tilted using a single-cycle acceleration trajectory in each of 100 trials. We selected a 5 s motion, with the majority of frequency content at 0.2 Hz, because prior work shows contributions of both the otolith organs sense of tilt angle and semicircular canals' sense of angular rotation at this frequency. Motion amplitudes were selected by a 3-down, 1-up adaptive staircase. Thresholds were estimated by fitting a cumulative Gaussian psychometric curve to subject response. A positive, linear correlation across subjects was found between manual control variability and vestibular thresholds ($p<0.01$) in the 1.0 G baseline. This suggests that sensory precision is a limiting factor in manual control performance. Additionally, manual control performance was 12.7% lower in 1.33 G ($p<0.05$) and 37.5% higher in 0.5 G ($p<0.05$), as compared to 1 G. The increased variability in 0.5 G may be due to decreased otolith shear force causing a decrease in sensitivity to tilt angles, with the converse occurring in 1.33 G.

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Poster

714. Balance, Posture, and Orientation

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Support: NIH/NIDCD R01-DC014924

R03-DC013635

Title: Does decision-making reflect high-pass filter contributions?

Authors: *D. M. MERFELD[1], F. KARMALI[2], T. LEEDER[2], K. LIM[2];
[1]Otol & Laryngol, [2]Harvard Med. Sch., Boston, MA

Abstract: Decision-making is a crucial aspect of cognition that impacts a broad range of fields. Recent vestibular threshold data showed a pattern of increasing thresholds when the frequency of applied single cycles of sinusoidal acceleration fell below about 1 Hz (1-4). While both perceived rotation measured via magnitude estimation (5) and peripheral transduction (6) show similar high-pass filter characteristics, the variation of thresholds as a function of frequency showed a much higher cut-off frequency (i.e., much lower time constant) than either perceived

rotation or peripheral transduction. These threshold variations as a function of frequency have been hypothesized to be consistent with the contributions of a high-pass filter (i.e., a neural mechanism that filters out low frequency signals) as part of the decision-making process (7-8). But at least two other related mechanisms - the addition of an urgency signal (9) and decision-boundaries that decrease with the passing of time (10) - might contribute to this observed threshold pattern. To test the contributions of these different hypothesized mechanisms, we chose to perform binary fixed-duration forced-choice tasks while rotating subjects about earth-vertical. Specifically, we varied the motion trajectory shape while maintaining the stimulus duration constant at 2 s with the peak stimulus velocity always occurring 1 s after the beginning of the motion. By maintaining stimuli at a constant duration, the impacts of an urgency signal and reductions in decision boundaries would be expected to be similar for different stimulus trajectories (i.e., be independent motion trajectory shape). In contrast, the high pass filter hypothesis forecasts predictable threshold variations as the shape of the motion trajectory is varied. To test these model predictions, we utilized different stimulus trajectory shapes, including bell-shaped velocity trajectories, triangular velocity trajectories, and parabolic velocity trajectories. We found that the thresholds were consistently higher for triangular velocity trajectories than for bell-shaped velocity trajectories. These results are consistent with the contributions of a high-pass filter to the decision-making process. (1) Grabherr, 2008 Exp Brain Res 186: 677-681. (2) Soyka, 2011 Exp Brain Res 209: 95-107. (3) Coniglio, 2014 J Assoc Res Oto 15: 305-317. (4) Valko, 2012, J. Neurosci, 32:13537-42. (5) Bertolini, 2011, J Neurophys 105: 209-223. (6) Goldberg, 1971 J Neurophys 34:635-660. (7) Merfeld, 2016, J Neurophys 1:39-59. (8) Lim, 2012, Exp Brain Res 222:303-320. (9) Churchland, 2008, Nat Neurosci. 11:693-702. (10) Drugowitsch 2012, J. Neurosci 32:3612-28.

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Poster

714. Balance, Posture, and Orientation

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Program#/Poster#: 714.14/JJ16

Topic: D.07. Vestibular System

Support: EU FP7 604063 HealthPAC

Title: Perception of the visual vertical during linear self-motion

Authors: ***A. POMANTE**, L. P. J. SELEN, W. P. MEDENDORP;
Donders Inst. for Brain, Cognition and Behaviour, Radboud Univ. Nijmegen, Nijmegen, Netherlands

Abstract: The vestibular system provides information for spatial stability and the percept of verticality. A stationary subject, seated upright, can align the orientation of a visual line to the direction of gravity with an accuracy better than 1 degree. A complexity arises when subjects are in motion. Because the otoliths sense the gravito-inertial force, they cannot distinguish gravitational and inertial accelerations. As a consequence, prolonged linear acceleration of the head can be interpreted by the brain as a tilt, even though the head does not rotate. In this study, we tested the dynamics of this illusion by probing the percept of the visual vertical (subjective visual vertical, SVV) during passive linear head motion. Human subjects were sinusoidally translated (f=0.4 Hz, 3.5 m^2/s peak acceleration, 60 cm peak-to-peak displacement) along an inter-aural axis using a vestibular sled. While they kept fixation on central body-fixed light, a line was briefly flashed (5 ms) at different phases of the motion. Using a psychometric approach, subjects judged the orientation of this line relative to gravity. So far, we have tested 5 subjects. Their results show a phase- dependent modulation of the SVV, with a stronger bias of the SVV at peak acceleration than at peak velocity. These findings provide evidence that the SVV is modulated by self-motion signals, presumably reflecting the brain's interpretation of the otolith signal. Our observations are consistent with the prediction from the modeling work of Laurens and Droulez, 2007 [1]: the vestibular canals and otolith signals are integrated in a statistically optimal fashion reproducing the rules of Bayesian inference.
References:
[1] Laurens J, Droulez J. Bayesian processing of vestibular information. Biol Cybern. 2007; 96:389-404.

Disclosures: A. Pomante: None. **L.P.J. Selen:** None. **W.P. Medendorp:** None.

Poster

714. Balance, Posture, and Orientation

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Program#/Poster#: 714.15/JJ17

Topic: D.07. Vestibular System

Title: Modulation of egocentric spatial frames of references by vestibular

Authors: *N. ABEKAWA[1,2], E. FERRE[3], M. GALLAGHER[2], H. GOMI[1], P. HAGGARD[2]; [1]NTT Communication Sci. Labs., Kanagawa, Japan; [2]UCL, Inst. of Cognitive Neurosci., London, United Kingdom; [3]Royal Holloway, Dept. of Psychology, London, United Kingdom

Abstract: Judging the position of external objects relative to the body is essential for interacting with environments. For instance, a tennis player has to quickly select a forehand or backhand shot based on the location of the ball in respect of the body. Thus, *egocentric* spatial

representations must be processed rapidly for successful control of actions. Clinical reports have suggested vestibular contributions to egocentric spatial representation. However, most of these studies estimated the perceptual "subjective center" of the body. It remains unclear whether vestibular-based egocentric representations also contribute to on-line selection of rapid actions. Importantly, vestibular signals could also affect visual perception and motor execution. This raises further questions regarding which processing stages along the visual-motor processing chain are effectively modulated by vestibular signals. To address this issue, we systematically explored how vestibular inputs contribute to rapid vision-body-motor processing. A visuomotor task was combined with low intensity Galvanic Vestibular Stimulation (GVS, 1mA square-wave). L-GVS (Left anode and right cathode), R-GVS (right anode and left cathode), or sham-GVS were delivered while participants judged whether a briefly-flashed visual stimulus was located to the left or right of their body-midline. They had to press as fast as possible a button located on their left or right side accordingly. To directly examine the effect of GVS on effector selection, the task was performed with hands uncrossed or crossed in different blocks. The results showed that GVS polarity influenced the subjective perception of body midline: L-GVS biased the subjective body midline toward the left, while R-GVS induced a bias in the opposite direction. This bias was not modulated by crossing the hands, ruling out explanations based on GVS affecting the effector selection processes. Explanations based on visual localization were also ruled out in an additional control experiment. Taken together, our results indicate that egocentric representation is strongly modulated by vestibular signals without the actual motion of the body or head. The findings support a model of egocentric representation centered on the body midline, and implemented by the balance between sensory signals in the left and right hemispheres. The vestibular systems *calibrate* an egocentric spatial frame, which allows on-line action selection for rapidly interacting with the external environment.

Disclosures: **N. Abekawa:** None. **E. Ferre:** None. **M. Gallagher:** None. **H. Gomi:** None. **P. Haggard:** None.

Poster

715. Vision and Eye Movements

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 715.01/KK1

Topic: D.08. Visual Sensory-motor Processing

Support: NSF CISE #1566621

Title: MT neurons preserve visually selective signals across eye movements

Authors: *A. AKBARIAN AGHDAM[1], M. PARSA[2], N. NATEGH[1], B. NOUDOOST[3]; [1]Electrical and Computer Engin., Montana State Univ., Bozeman, MT; [2]Computer Science, Montana State Univ., Bozeman, MT; [3]Cell Biol. and Neurosci., Bozeman, MT

Abstract: Our perception of visual world stays stable despite substantial retinal image motion due to the thousands of saccades that we make each day. Research suggests that higher-order prefrontal and parietal areas contribute to the maintenance of visual stability across saccades. Because higher-order areas lack the visual feature selectivity of early sensory areas, it has remained unclear how visual representations are preserved during the disruption of retinal input due to eye movements. We measured the receptive fields (RFs) of neurons in the middle temporal (MT) area of two rhesus monkeys before, during, and after a visually guided saccade using brief pseudo-random flashing probes. The flashing probes appeared on the screen in a grid covering both the pre- and post- saccadic RFs, as well as the initial fixation point and the saccade target. Each probe appeared at pseudo-random locations for 7ms enabling us to precisely map the neuron's RF around the brief period of saccade execution. We found that MT neurons exhibit a persistent visual activity during eye movements: when the flashing probe appears within the neuron's RF very near to the time of the saccade onset (-10 to +10 ms) some MT neurons exhibit a response that starts with the usual latency of the MT neuron but persists across the saccade and even after the eyes have landed. Such persistent activity during eye movements could serve as an extrastriate mechanism for preserving visual information across saccades. This activity could contribute to perceptual stability across saccades, maintaining the pre-saccadic representation until the post-saccadic one is formed. In order to examine this idea, we employed an explicit probabilistic model in the generalized linear model (GLM) framework, which enables us to create a readout of the visual scene during eye movements based on MT spike trains. This encoding and readout modeling framework suggests that the persistent activity is necessary for trans-saccadic integration of the visual representation.

Disclosures: A. Akbarian Aghdam: None. **M. Parsa:** None. **N. Nategh:** None. **B. Noudoost:** None.

Poster

715. Vision and Eye Movements

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Topic: D.08. Visual Sensory-motor Processing

Support: Alfred P. Sloan Foundation BR2014-098

NIH R01-EY025648

Title: Memory for retinotopic locations is more accurate than memory for spatiotopic locations, even when intending to reach.

Authors: *A. SHAFER-SKELTON, J. D. GOLOMB;
Dept. of Psychology, The Ohio State Univ., Columbus, OH

Abstract: In order to successfully interact with objects, we must maintain stable representations of their locations in the world. However, their images on our retina may be displaced several times per second by large, rapid eye movements. Are we able to form a seamless world-centered (spatiotopic) representation of objects' locations across eye movements? Surprisingly, Golomb & Kanwisher (2012 PNAS) found that memory for an object's location is more accurate and precise in gaze-centered (retinotopic) than world-centered (spatiotopic) coordinates, and that the accuracy of spatiotopic but not retinotopic memory deteriorates with each eye movement. This suggests that the native coordinate system of visual memory is retinotopic. We used a human behavioral paradigm to investigate whether the intention of acting on an object's location could improve memory for its spatiotopic location. Twelve participants were asked to remember a spatial location across a short delay. During the delay, they completed a variable number of eye movements (0-2). Participants completed four versions of this task in separate sessions (with session order counterbalanced across participants); they reported either the retinotopic or spatiotopic location, either using a mouse to click on the remembered location (as in Golomb & Kanwisher, 2012) or using their finger to interact directly with the touchscreen. We replicated Golomb & Kanwisher's original results. Critically, we found the same pattern of results in the reaching task. These results further support the hypothesis that spatial memory is natively retinotopic; we found no evidence that the intention to act on an object improves spatiotopic memory across saccades. How, then, do we successfully act on objects in the world? One possibility is that the rich information contained in visual scenes could serve as landmarks that could facilitate the creation of more seamless spatiotopic representations. Further research could explore whether and what types of object and/or scene information might contribute.

Disclosures: A. Shafer-Skelton: None. **J.D. Golomb:** None.

Poster

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Program#/Poster#: 715.03/KK3

Topic: D.08. Visual Sensory-motor Processing

Support: NSF CISE #1566621

Title: Modeling perisaccadic visual representation in MT neurons by incorporating population-level information

Authors: *K. NIKNAM[1], A. AKBARIAN AGHDAM[1], B. NOUDOOST[2], N. NATEGH[1]; [1]Electrical & Computer Engin. Dept., [2]Dept. of Cell Biol. & Neurosci., Montana State Univ., Bozeman, MT

Abstract: How the brain maintains the stability of visual perception across saccades is one of the oldest questions in systems neuroscience; accurately characterizing visual responses in this perisaccadic period is an important step towards understanding how the visual world is represented during saccades. We recently developed a probabilistic model in the Generalized Linear Model (GLM) framework to characterize and predict the pre-, trans-, and post-saccadic responses of single middle temporal (MT) neurons at the level of single-trial spike trains. Here we report an extended form of the model incorporating statistical dependencies of MT responses, such as the correlations between neurons as well as the local field potentials (LFPs). The responses of multiple neurons within the MT cortex of macaque monkeys were simultaneously recorded using linear array electrodes. The animals were performing a visually-guided saccade task while a set of probes flashed in pseudorandom positions around the estimated receptive field (RF) of the MT neurons during fixation and saccade execution. Probe presentations were brief, enabling us to characterize the spatiotemporal RF changes with a temporal resolution higher than the saccade execution time. The responses of these neurons to drifting Gabor gratings were also recorded during a fixation task to determine the motion sensitivity of individual neurons as well as the correlations between neurons. To characterize the time-varying information carried by MT neurons, we fit the spiking response of an MT neuron to an extended GLM, consisting of a set of linear kernels to mimic the spatiotemporal properties of the MT neurons' RFs, a set of saccade kernels to account for the changes in stimulus sensitivity induced by eye movements, an offset kernel to account for time-varying baseline activity relative to the saccade, a post-spike kernel to account for dependencies on spiking history (such as refractoriness, burstiness, and adaptation), and finally, a set of coupling kernels to account for correlations between neurons. In addition to visual information, the input to this extended model includes the spiking response of other neurons and the LFP, which may provide additional information about brain state or cognitive factors. The model allows us to predict the responses of MT neurons to a variety of visual stimuli around the time of the saccade. This in turn enables us to test the contribution of specific perisaccadic changes in neuronal responses to the representation of the visual scene during eye movements.

Disclosures: K. Niknam: None. **A. Akbarian Aghdam:** None. **B. Noudoost:** None. **N. Nategh:** None.

Poster

715. Vision and Eye Movements

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Program#/Poster#: 715.04/KK4

Topic: D.08. Visual Sensory-motor Processing

Title: Modulation of binocular rivalry by stimulus complexity, arousal and caffeine

Authors: *A. C. O. BOATENG[1], D. E. FLEMING[2], S. C. STEFFENSEN[2];
[1]Brigham Young Univ. Hawaii, Laie, HI; [2]Brigham Young Univ., Provo, UT

Abstract: Abstract: The most common form of perceptual rivalry is visual system binocular rivalry (BR), which has been known since the 18th century. Binocular rivalry is characterized by perceptual alternation (BRPA), where perception alternates between stimuli presented separately to the eyes. Binocular rivalry appears to be a stochastic neural process, but under a modicum of dynamic conscious control. While the brain substrate of BRPA is not clearly identified, fMRI studies suggest that it originates in brainstem structures (Pettigrew et al., 2001), but involves neural competition at multiple levels of the visual pathway (Freeman et al., 2005; Wilson et al., 2003). The aim of this study was to determine if BRPA can be modulated by stimulus complexity, arousal and the psychostimulant caffeine. In addition, we also evaluated the effects of caffeine on color afterimages. Experiments were performed in 306 college-age (20-27 yrs) male and female subjects with a program that logged keyboard presses whenever the subject indicated the perception of red or blue arrows vs red or blue generic faces presented to the eyes separately via superimposed images through red/blue 3D anaglyph glasses in a 2 min session. There was no significant difference in BRPA between male and female subjects (28.2 ± 0.8 alternations/min in males vs 29.4 ± 1.1 alternations/min in females). BRPA was significantly slower when the subjects viewed the images of faces vs arrows ($p<0.01$), and significantly faster following hyperventilation for 2 min ($p<0.05$) or 30 min after the consumption of 1 mg/kg caffeine ($p<0.05$). In a separate study, undergraduate students (n=31, age range: 18-24 years) with a history of regular caffeine consumption were tested on opponent color processing (green/red, magenta/cyan, blue/yellow) and BR (arrows vs faces) tasks after three levels of conditions: placebo, low (1mg/kg) and high (5mg/kg) dose of caffeine. The results show a biphasic response with low and high dose caffeine as well as a wavelength sensitivity, with blue-yellow afterimages enduring longer at high dose $F(2,180) = 31.69$, $p < 0.01$, $\eta^2 = 0.26$ and increase in perceptual alternation time $F(4,556) = 6.77$, $p < 0.05$, $\eta^2 = 0.05$ with an overall interaction effect between conditions and color type $F(4,180) = 8.40$, $p < 0.01$, $\eta^2 = 0.15$. Taken together, these findings provide strong evidence that BR is modulated by the complexity of the stimulus and the degree of physiological arousal and that psychostimulants increase the rate of perceptual switching but have a dose-related biphasic effect on the endurance of color afterimages. Studies are ongoing to elucidate electroencephalographic correlates of BR.

Disclosures: **A.C.O. Boateng:** None. **D.E. Fleming:** None. **S.C. Steffensen:** None.

Poster

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Title: Parahippocampal gyrus neurons in the monkey respond to stimuli in the entire visual field except for the foveal region.

Authors: ***M. SEMEWORK**[1], M. E. GOLDBERG[2];
[1]Dept. of Neurosci., Columbia Univesity, New York, NY; [2]Columbia Univ., New York, NY

Abstract: When we enter a new environment we immediately and automatically establish a gist memory of that environment, so that we can, with our eyes closed, point at or look at salient objects in the new space. Patients with parietal cortical lesions can locate objects in the room with their eyes open, but cannot do so with their eyes closed (Levine, 1985). Place cells in the hippocampus (O'Keefe & Dostrovsky, 1971) represent an environmental memory, and neurons in the FEF (Umeno & Goldberg, 2001) and LIP (Steenrod & Goldberg, 2009) demonstrate this environmental memory. One pathway by which the spatial memory signal of the hippocampus could reach the parietal cortex is by way of the parahippocampal gyrus (PHG), area TF. Here we sought to evaluate the spatial properties of neurons in PHG in order to understand what they might contribute to the process of spatial memory.We recorded the activity of neurons in PHG adjacent to visual area TE in an awake Rhesus monkey performing fixation, memory- and visually-guided delayed saccade tasks (electrodes located at +2 AP, +15 ML stereotactically). We found a class of neurons with very fast visual latencies (50-70 ms). In the area in which they were found, every cell in a vertical penetration exhibited the same properties. The cells respond to stimuli throughout the visual field, as far as we could measure (25^0 in the periphery) with the salient exception of the 2.5-5^0 around the center of gaze. The onset of the visual response over trials jitters a few milliseconds (+/- 5 ms). The cells responded to visual stimuli of various sizes, a tiny dot to several degrees diameter - with larger probes evoking larger (higher peak, and a little longer) responses. The cells were not selective for color, orientation, or shape, and gave

equal responses from either eye. There is no systematic variation in the maximums, means and latencies of the visual responses when probes are presented ipsi/contraletrally. They did not distinguish between visual probes in the fixation task and saccade targets, except when the monkeys made a visually guided saccade the saccade target, now in the fovea, suppressed the response.The role of such large receptive fields with a hole around the fovea might be to suppress the response of the PHG to unattended stimuli. The majority of the neurons have 'ambiguous' waveforms according to the Shin and Sommer (2012) criteria for frontal eye field neurons, circumstantial evidence that they might be inhibitory neurons.

Disclosures: **M. Semework:** None. **M.E. Goldberg:** None.

Poster

715. Vision and Eye Movements

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Title: Perceptually-driven rightward saccades for detection of lateralized visuospatial attention system

Authors: ***M. SANEFUJI**[1,2], H. YAMASHITA[3], Y. SAKAI[1], D. KATSUKI[3], S. AKAMINE[1], Y. SHIOTSUKA[4], Y. ISHIZAKI[1], K. IRAMINA[4], T. HARA[5], K. YOSHIDA[3];
[1]Pediatrics, [2]Res. Ctr. for Envrn. and Developmental Med. Sci., [3]Child Psychiatry, [4]Syst. Life Sci., Kyushu Univ., Fukuoka, Japan; [5]Fukuoka Children's Hosp., Fukuoka, Japan

Abstract: [Introduction] Neuroimaging and psychological studies in neurologically normal adults have demonstrated right-lateralized visuospatial attention system. However, the lateralization remains unknown during early childhood because of methodological constraints. To estimate the lateralization for infants, we have invented a brief task that elicits initially a perceptual-driven saccade and subsequently predictive saccades. To check the task's significance, we examined the association of the task with line bisection test, a lateralization test for perceptual attention, in adult subjects. [Methods] Participants were 57 right-handed adults. A

target steps between two horizontal fixed locations at a fixed time interval. The first saccade was leftward for half of the subjects and rightward for the remaining half. They were just instructed to follow the target. They did not know the timing of the target appearance at the first saccade but, in the following saccades, they began to anticipate the appearance of the target and more rapidly make a saccade towards the expected location. Saccades were monitored using a remote eye-tracker and the latencies were analyzed separately for rightward and leftward saccades. We hypothesized that the first saccade is perceptually driven while the following predictive saccades are more internally generated and less perceptual. Thus, the latency of the first saccades subtracted by that of the following saccades could represent the processing time required for the perception of the target presented in a hemi-field. [Results] As expected, latency rapidly decreased from the first to the following saccades. In line bisection test, participants showed variable biases, but as a whole, a slight left deviation. For rightward saccades, a longer subtracted latency corresponded to a greater deviation toward the left in line bisection. For leftward saccades, there was no such correlation. [Discussion] The longer subtracted latency for rightward saccades indicates that more time is required for the perception of the target in the right-field. Such individuals could have less left hemispheric function than right function, and perceptually underestimate the right-side of the line, bisecting the line with left deviation. In contrast, the absent correlation for leftward saccades may be accounted for by the classical theory that the right hemisphere is not directionally biased compared with left hemisphere. This brief task will reveal whether the hemispheric lateralization of visuospatial function already exists during early childhood.

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Poster

715. Vision and Eye Movements

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Topic: E.01. Eye Movements

Support: R01 MH-055687

Title: Electrocorticographic correlates of saccades

Authors: ***A. GELLER**[1], J. BURKE[2];
[1]Neurol., NYU, New York, NY; [2]Neurosurg., UCSF, San Francisco, CA

Abstract: Cortical control is known to be critical in driving horizontal gaze. The frontal eye fields have been studied both in non-human primates, where they have been localized to Brodmann area 8, and in humans, where there remains some debate regarding their precise localization. Cortical control for vertical gaze, if present, remains obscure; the lower motor neurons for cranial nerves 3 and 4 are known to be triggered by the Rostral interstitial nucleus of the medial longitudinal fasciculus (riMLF) with support from the Interstitial nucleus of Cajal (iNC). We examined the electrocorticographic correlates of both horizontal and vertical conjugate eye movements in 90 epilepsy patients undergoing invasive monitoring, who performed a task engaging the extraocular muscles in ballistic eye movements as well as smooth pursuit. We found that both tasks activated visual cortex. The horizontal gaze task preferentially activated superior temporal cortex (BA 41 and 22), while the vertical gaze task preferentially activated posterior temporal cortex (BA 37).

Disclosures: **A. Geller:** None. **J. Burke:** None.

Poster

715. Vision and Eye Movements

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Topic: E.01. Eye Movements

Title: The medial ventro-thalamic microconnectomics: cells implicated in a bimodal motor circuit

Authors: *T. VEGA-ZUNIGA, D. TROST, K. SCHICKER, H. LUKSCH;
Technische Univ. Muenchen, Freising, Germany

Abstract: A common feature of the ventral thalamus of birds is the presence of a group of retino-recipient and somatosensory structures located adjacent to each other. This includes the intergeniculate leaflet (IGL), n. geniculatus lateralis pars ventralis (GLv), n. ventrolateral thalami (VLT) and n. intercalatus thalami (ICT). These structures have in common an absence of projections to the telencephalon, together with efferent projections to the pretectum, tegmentum and pontine targets. Although the general connectivity is known, detailed morphology and connectivity pattern is still elusive. Here, by means of intracellular filling technique, we focused on two neural structures, namely, the retino-recipient neuropil (ne) of the GLv, and the adjacent somatosensory nucleus ICT. We found that the GLv-ne cells showed two different neuronal types: projection cells and horizontal interneurons. The projection cells showed a variable morphology and dendritic arborization with axons that target the n. lentiformis mesecephali (LM), griseum tectale (GT) and ICT. The horizontal cells showed a widespread medio-lateral

dendritic arborization throughout the retino-recipient GLv-ne. The ICT cells on the other hand, showed multipolar somata with a projection pattern that targets the GLv-li, n. laminaris precommissuralis (LPC), GT and TeO. Together, these results add more complexity to the tangled connectivity pattern so far described between the GLv, ICT, LM, GT and TeO. Interestingly, the implication of some of these neural structures in visuomotor and somatosensory functions suggests that these nuclei may form part of a bimodal circuit involved, among other tasks, in the generation/modulation of saccades, gaze control and space perception.

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Poster

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Topic: E.01. Eye Movements

Support: NIH Grant R01 NS-058659

Title: Gaze coordination with strides during walking in the cat

Authors: K. M. I. CHU[1], H. N. ZUBAIR[1], J. L. JOHNSON[1], T. J. RIVERS[2], *I. N. BELOOZEROVA[1];
[1]Neurobio., Barrow Neurolog. Inst., Phoenix, AZ; [2]Dept. of Ecology & Evolutionary Biol., Univ. of Kansas, Lawrence, KS

Abstract: Vision plays a crucial role in guiding locomotion, especially in complex environments. However, the relationship between gaze behaviors and phases of the stride is not well understood. We investigated gaze behaviors of cats walking in four conditions: (1) on a flat surface in darkness or (2) in the light, (3) on a horizontal ladder or (4) on a pathway cluttered with small stones. We recorded vertical and horizontal eye movements and 3-D head and forelimb movements, and calculated where the cat's gaze intersected the walkway. Movements of the gaze intersect point were classified into four categories: gaze shifts away from the cat, gaze shifts toward, fixations, and constant gaze. Occurrences of gaze behaviors were correlated with phases of the stride.
We found that even during walking on the flat surface in the dark, most gaze behaviors were coordinated with strides. Toward gaze shifts typically began just before the initiation of a forelimb's swing or during the first 1/3 of the swing and ended in the middle of swing phase. Away gaze shifts began throughout the second half of swing of each forelimb and ended when both forelimbs were in stance. Fixations peaked in the middle of each forelimb's swing phase,

roughly between the end of gaze shifts toward and beginning of gaze shifts away. Constant gaze had no clear relation to strides.
Introducing light markedly increased synchronization of gaze behaviors with strides. Toward gaze shifts only started in the first 1/3 of swing phase, while away gaze shifts started in the last 1/3 of this phase. Fixations occurred throughout most of each forelimb's swing phase, except near the end when constant gaze dominated. Few fixations were seen during the transition from swing to stance.
Walking on the horizontal ladder requires vision (Beloozerova and Sirota, 2003). On the ladder, gaze behaviors were even more synchronized with strides. Toward gaze shifts were confined to the beginning of swing phase. They were followed by fixations, which in turn were followed by away gaze shifts. Constant gaze was also highly synchronized with strides in two of three cats, occurring during the transitions from swing to stance phases of each forelimb.
Gaze behaviors during walking on a surface cluttered with many small stones were similar to those observed on the ladder. The most prominent difference was that constant gaze occurred slightly earlier in the cycle, at the end of the swing phase.
We concluded that gaze is synchronized with strides even when vision is unavailable or unnecessary for successful walking. This synchronization increases when vision is available and further increases on a complex terrain when accurate stepping is required.

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Poster

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Topic: E.01. Eye Movements

Support: CIHR 394524

Title: Visual saliency response in the superficial and intermediate superior colliculus

Authors: *J. Y. KAN[1], B. J. WHITE[1], L. ITTI[2], D. P. MUNOZ[1];
[1]Ctr. for Neurosci. Studies, Queen's Univ., Kingston, ON, Canada; [2]Computer Sci., USC, Los Angeles, CA

Abstract: The superior colliculus (SC) is a phylogenetically old midbrain structure that plays a central role in vision, attention, and orienting. The SC has visual representations in the superficial-layers (SCs), and sensorimotor representations linked to the control of eye

movements and attention in the intermediate-layers (SCi). Cognitive and computational neuroscience postulates the existence of a visual saliency map that guides visual orienting towards the most visually conspicuous stimuli, and a priority map that combines bottom-up saliency and top-down relevance to allow internal processes such as goals and expectations to also guide behavior. We hypothesize that the SCs embodies the role of a bottom-up saliency map while the SCi embodies the combined priority map. To test this hypothesis, we compared SCs and SCi activity in response to task-irrelevant salient stimuli. Monkeys viewed a wide-field arrangement of stimuli (210 radially-arranged items spanning ~40-50deg) extending beyond the classic receptive field (RF). The stimuli were oriented color-bars (~0.4x1.2deg) that formed a perceptual "pop-out" array the monkeys had to ignore; i.e., reward was contingent upon maintaining gaze on central fixation. We compared visual representations in SCs and SCi when 1 to 4 salient pop-out stimuli appeared equally spaced within the array. We also compared this to an array of homogenous items with no pop-out. Only SCs neurons showed a reliable preference for the visually salient but goal irrelevant pop-out stimulus. Also, this representation in the SCs was the same in the presence of 1 to 4 pop-out items. It is important to note that the pop-out stimuli in the present task was goal IRRELEVANT. In the SCi, we only observed a response to a single salient pop-out stimulus; this response was absent once there were 2 or more salient pop-out items. Next, we will examine how activity in the SCs and SCi changes when the salient stimuli become also goal RELEVANT by being the target of a saccade that will lead to differing amount of reward.

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Poster

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Topic: E.01. Eye Movements

Support: AMIDEX Rising Star Chair

ANR @Raction

Title: Functional connectivity within and between the oculomotor and the face-perception networks in the human brain when looking towards or away from faces

Authors: *M.-H. GROSBRAS[1], B. NAZARIAN[2], E. SALVIA[1];
[1]Lab. de Neurosci. Cognitive- CNRS, [2]Inst. des Neurosciences de la Timone- CNRS, Aix Marseille Univ., Marseille, France

Abstract: Faces attract and retain attention more than other objects. Here we used functional magnetic brain imaging (fMRI) to investigate how the functional coupling between oculomotor brain regions and visual brain regions is modulated when participants look towards or away visual stimuli belonging to different categories. We scanned 23 healthy adults volunteers in an event-related design intermixing pro- and antisaccades towards or away either faces or cars. Each individual completed six runs of 40 trials. We also acquired a high resolution We identified regions of the oculomotor network and of the face- perception network using coordinate-based meta-analyses of brain imaging studies. We For each functional region we defined a sphere of 6 mm radius around the coordinate of maximum likelihood activation and computed the average signal across the voxels witin the spahere. Then we used psychophysiological interaction analyses to test how the different conditions affect the functional coupling between nodes within and between these two networks. We observed increased functional connectivity during antisaccades away from faces compared to cars (i) between the frontal eye-field (FEF) and posterior oculomotor regions. (ii) between the insula and the amygdala and middle temporal temporal gyrus (i.e within the face network); (iii) and between the right frontal eye field and a region of the left inferior frontal gyrus.
These data suggest that the functional connectivity within the oculomotor network is increased in presence of a socially salient stimulus such as a face. Likewise the functional organization of the face network and its interaction with the FEF is modulated by the oculomotor task, but mainly with respects to the nodes that are more commonly associated with the perception of emotional facial expressions. These data highlight specific neural bases to the increased attentional demand induced by orienting towards or away from faces as compared to other objects.

Disclosures: **M. Grosbras:** None. **B. Nazarian:** None. **E. Salvia:** None.

Poster

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Topic: E.01. Eye Movements

Support: CNRS

Title: New perspectives brought by the study of saccades toward an accelerating target

Authors: ***A. TASSOU**, L. GOFFART;
Inst. De Neurosciences De La Timone, Marseille, France

Abstract: An object moving in the peripheral visual field triggers a saccade which orients the gaze toward its location, even though the target-evoked retinal activity consisted of a brief and transient streak. How does the brain transform time-varying signals into a saccade landing position? A recent study showed that this transformation depended upon the target location, the speed of its motion and the rate of change in speed. When the saccades were made in response to a transient target that accelerated (from 0 to 40 deg/s) in the peripheral visual field, a linear relation was found between the landing positions of saccade and their response times (delay between target onset and saccade end). This relation had a slope which was close to the terminal speed of the target (Quinet & Goffart 2015). We investigated further this dependency by testing in three head-restrained monkeys, the saccades made in response to a target which accelerated to a larger range of maximum speeds (from 20 to 180 deg/s). Like in the previous study, the saccades were initiated from a fixation target located straight ahead that the monkeys had to fixate for a variable interval. Then, a transient moving target (duration=200ms) appeared in the upper or lower visual field (16° above or below the horizontal meridian). The target moved horizontally to the left or to the right, and the monkeys' task was to look at the zone traveled by the target with barely any constraint on the accuracy of their response. As shown previously and in spite of the stealth of the target, the saccade endpoints were not randomly distributed; the horizontal landing position of saccades increased linearly with their response time. However, this linearity was observed up to a maximum. Beyond a delay of approximately 100 ms after the target offset, the horizontal landing position stopped increasing. Below this delay, the relation was linear and its slope was correlated with the terminal speed of the target. The increasing horizontal speed of the target led to discover an asynchrony between the horizontal and vertical components of saccades which had never been reported before: the horizontal component was delayed with respect to the vertical component with an average delay which tended to decrease with the target speed.
Our results suggest that within a bounded time window, the brain performs a derivative function (in the mathematical sense) in response to time-varying signals. The quadratic time function of target position is transformed into a linear function. Moreover, the asynchrony between the horizontal and vertical components of saccades toward accelerating targets calls into question the idea that a single goal command drives saccades.

Disclosures: **A. Tassou:** None. **L. Goffart:** None.

Poster

715. Vision and Eye Movements

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 715.13/KK13

Topic: E.01. Eye Movements

Support: Knight Templar Eye Foundation

Fight for Sight

Blind Children Center

Research to Prevent Blindness- Departmental Grant

Title: Visual search and fixational eye movements in amblyopia

Authors: *F. F. GHASIA[1], J. OTERO-MILLAN[2], A. SHAIKH[3];
[1]Cole Eye Inst., Cleveland Clin., Cleveland, OH; [2]Neurol., Johns Hopkins Sch. of Med., Baltimore, MD; [3]Neurol., Case Western Reserve Univ., Cleveland, OH

Abstract: Introduction Miniature eye movements such as microsaccades shift the image on the fovea and counteract visual fading. They also serve as an optimal sampling strategy while viewing complex visual scenes. The goal of our study was to assess visual search and microsaccade production in amblyopia. **Methods**Eye movements were recorded using infrared VOG during amblyopic and fellow eye viewing while the subjects identified picture differences. 24 amblyopes (mild =11;moderate= 9; severe=4) and 9 controls were recruited. **Results**Amblyopic subjects had comparable dwell time in the relevant interest areas during both fellow eye viewing (normal: 49.8%; mild: 48.4%; moderate: 45.9%; severe: 43.6%, ANOVA p=0.5) and amblyopic eye viewing conditions (normal: 49.8%; mild: 45.7%; moderate: 39.8%; severe: 47.3%, ANOVA p=0.5). Amblyopes were able to identify comparable picture differences during fellow eye viewing to controls (normal: 5.7; mild: 5.8; moderate: 5.2;severe: 4.3, ANOVA p=0.3). However, the ability to identify picture differences was diminished during amblyopic eye viewing condition (normal: 5.8; mild: 3.8; moderate: 2.7;severe: 0.8, ANOVA $p<0.05$). In amblyopes who were able to identify the picture differences the reaction time was increased during both fellow and amblyopic eye viewing condition(Fellow eye viewing: normal: 8.5 s; mild: 8.0 s; moderate: 14.9 s; severe: 11.9 s KW ANOVA p=0.03; Amblyopic eye viewing: normal:8.5 s; mild: 7.4 s; moderate: 16.7 s; severe: 11.3 s KW ANOVA p=0.01). There was a decrease in the frequency of microsaccades in amblyopes (normal:0.3 Hz, fellow eye viewing:0.2 Hz, amblyopic eye viewing:0.06Hz).**Discussion**The brain increases the rate of microsaccades to aid visual exploration. The relative increase in production of microsaccades while viewing crowded visual scene is diminished in amblyopes. These results suggest that alteration in micro-saccades could explain the difficulty in perceiving details of a complex picture evident as crowding phenomenon in amblyopia.

Disclosures: F.F. Ghasia: None. **J. Otero-Millan:** None. **A. Shaikh:** None.

Poster

715. Vision and Eye Movements

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Topic: E.01. Eye Movements

Support: NIH Grant R01 EY022928

Ocular Tissue Engineering and Regenerative Ophthalmology Fellowship (OTERO)

Title: Eye movements induced by patterned microstimulation of the frontal eye fields

Authors: *M. A. SMITH, R. O. KONECKY;
Ophthalmology, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: The frontal eye fields (FEF) are the region of cortex most closely linked to the generation of eye movements, receiving visual inputs and sending descending projections to subcortical regions that control saccades. FEF is functionally identified as the region of prefrontal cortex in which saccades can be generated by electrical microstimulation at low currents. Supra-threshold stimulation at a particular site produces a saccade with a characteristic direction and amplitude (Bruce et al, 1985). Raising the current above the threshold produces little change in the saccade, and extending the duration often produces repeated saccades with the same vector ("staircase saccades"). The relationship between microstimulation strength (current) and behavior has been used to deduce features of the functional organization of visual and motor cortex. Similarly, the behavior of animals under conditions in which both microstimulation and visual input are provided simultaneously has been used to study the integration of sensory activity by downstream targets. Here, we employed microstimulation in FEF at multiple sites simultaneously to determine how the output of FEF is summed to produce behavior. To investigate this question we microstimulated at multiple sites concurrently in the FEF of macaque monkeys. We used a linear array of 16 channels spaced 150 μm apart to microstimulate FEF with small amounts of current (15-150 μA). Using this experimental design, we tested the following hypotheses: (1) do the contributions of multiple neuronal populations in the FEF sum in a predictable way, (2) can their contributions be manipulated by varying the current into these inputs and (3) can their contributions be manipulated by varying the distance between inputs. We found that the direction of evoked saccades resulting from simultaneous stimulation of two contacts is equal to the average saccade vector evoked from stimulation of each constituent contact alone. We were able to shift the resultant saccade vector from one constituent saccade to the other by varying the amount of current on each contact. The ability to produce this graded response saturated at supra-threshold currents. Also, we found that greater inter-contact distances increased the amount of error between the vector average prediction and the actual saccade. Evaluating the effects of patterned microstimulation provides a richer

understanding of the complexities of the oculomotor system. More broadly, these data demonstrate how cortical inputs combine to produce complex outputs and serve as a foundation for the development of neural interfaces to influence perception and behavior with microstimulation.

Disclosures: **M.A. Smith:** None. **R.O. Konecky:** None.

Poster

715. Vision and Eye Movements

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Topic: E.01. Eye Movements

Support: NIH Grant EY10217

NIH Grant EY02162

Title: Eye movement recordings of spontaneous fusion loss in intermittent exotropia

Authors: ***J. C. HORTON**, D. L. ADAMS, J. R. ECONOMIDES;
Beckman Vision Ctr., Univ. California, San Francisco, CA

Abstract: Subjects with intermittent exotropia have normal visual acuity in each eye and intact stereopsis, but a large exophoria that results periodically in spontaneous loss of fusion. The factors that trigger breakdown of fusion are not well understood. No recordings have been made to characterize the type of movement made when the eyes diverge. To address this issue, we tested 10 subjects (mean age 31, range 12 - 61 years) with intermittent exotropia. They fixated a small central target rear-projected onto a tangent screen while the positions of their eyes were monitored with video eye trackers. A cover-uncover test was performed at unpredictable intervals by occluding either eye with a filter mounted on a pneumatic cylinder. The filter transmitted infrared light, so that the position of the covered eye could still be tracked. Between cover-uncover trials, while both eyes were viewing the fixation target, subjects experienced occasional episodes of spontaneous fusion loss. Outwards eye movements, occurring either spontaneously or after monocular occlusion, were compared. The mean exotropia was 17° (range 9 - 32°). Exotropia magnitude showed slight asymmetry, being an average of 14% larger with one eye covered compared with the other. Mean exotropia was nearly identical for spontaneous fusion loss and cover-induced trials. The difference in peak velocity of outwards eye movements occurring spontaneously or after occlusion was 1.1°/sec ($p = 0.59$). In subjects with larger exodeviations, peak velocities tended to be higher. In some subjects, additional testing was

performed by arranging two targets in depth, along the line of sight of either eye. Subjects were asked to shift fixation from the near to the far target. Target distances were chosen to evoke an eye movement that matched the amplitude of the subject's exotropia. The resulting divergence eye movement resembled the eye movement recorded during spontaneous loss of fusion. This similarity suggests that intermittent exotropes make a divergence eye movement when their ocular alignment breaks down. This divergence eye movement is not significantly affected by occluding the drifting eye. Intermittent exotropia appears to be a disorder of the divergence eye movement system.

Disclosures: **J.C. Horton:** None. **D.L. Adams:** None. **J.R. Economides:** None.

Poster

715. Vision and Eye Movements

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Johns Hopkins Science of Learning Institute Fellowship

Title: Sensory prediction errors during saccade adaptation drive cerebellar complex spikes and learning

Authors: ***D. J. HERZFELD**[1], Y. KOJIMA[2], R. SOETEDJO[2], R. SHADMEHR[1];
[1]Dept. of Biomed. Engin., Johns Hopkins Univ., Baltimore, MD; [2]Natl. Primate Ctr., Univ. of Washington, Seattle, WA

Abstract: The hypothesis of cerebellar learning suggests that adaptation is driven by prediction errors, communicated via climbing fibers to Purkinje cells (P-cells), resulting in complex spikes (CSs). In this framework, presence of a CS results in synaptic plasticity which changes the simple spike (SS) activity of P-cells and subsequently behavior. Recent attempts to validate this hypothesis during short-term saccade adaptation have failed to find a link between P-cell SS activity and movement parameters. Therefore, adaptation, which entails a change in this

encoding, has been even more difficult to address. However, we recently found that the combined responses of P-cells, organized by their CS tuning properties, reliably predict the real-time velocity of eye movements. Using this result, we have begun to address the question of adaptation.
We trained monkeys to produce saccades to visual targets while recording single-unit P-cell activity from the oculomotor vermis (OMV). In every trial, the monkey made a saccade to a random target. However in some trials, we jumped the target, producing a foveal error.
We found that an unexpected visual event consistently modulated the probability of a CS (Pr[CS]). During fixation, presentation of a random target produced a CS with a probability that was cosine tuned as a function of the foveal direction of the target. Once the saccade was completed, the same P-cell exhibited a cosine tuned Pr[CS], now with respect to foveal direction of error. That is, in both periods of time, a visual sensory prediction error (target location with respect to the fovea) modulated Pr[CS]. Therefore, the complex spikes have a directional preference for sensory prediction errors, regardless of whether the sensory event is associated with a previous movement or not.
Finally, we asked whether Pr[CS] affected SS responses during gain-down adaptation. We functionally organized the P-cells into microclusters wherein all P-cells shared a common CS tuning, and then computed a SS population response. When the post-saccadic prediction error occurred in the preferred CS direction of the P-cells, the SS population activity decreased and accurately predicted the gain of the saccade (R2=0.86). However, when the error occurred in the anti-preferred CS direction, the population response showed little or no changes.
Our results suggest that occurrence of a complex spike is associated with a sensory prediction error, even when that error is not associated with a previous movement. However, when the error occurs following a movement, then the increased Pr[CS] coincides with a reduction in the population SS response, driving plasticity during saccade adaptation.

Disclosures: **D.J. Herzfeld:** None. **Y. Kojima:** None. **R. Soetedjo:** None. **R. Shadmehr:** None.

Poster

715. Vision and Eye Movements

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Topic: E.01. Eye Movements

Support: NIH Grant EY022854

Title: Interceptive saccades revisited: a comparison of saccades to stationary and moving targets

Authors: K. J. MOHSENIAN[1], B. STEINBERG[2], A. L. CECALA[2], *N. J. GANDHI[1]; [1]Dept. of Bioengineering, Univ. of Pittsburgh, Pittsburgh, PA; [2]Dept. of Biol., Elizabethtown Col., Elizabethtown, PA

Abstract: Natural environments are dynamic and filled with sensory information that could be used by an organism to guide behaviors necessary for survival. Animals are able to extract relevant information from their environment by aligning their specialized sensory apparatus (e.g. the retinal fovea) with stationary and moving objects. Rapid eye movements have been used to study sensory, motor, and cognitive processes in primates, but most of this research has emphasized the use of stationary targets. Under these circumstances, the metrics and kinematics of saccades have been well characterized (Leigh & Zee, 2015); however, there have been relatively few in-depth descriptions of horizontal, vertical, and oblique interceptive saccades using a large range of target speeds and directions. The current abstract reports data collected from two rhesus monkeys and five human subjects who performed a delayed saccade task in which the delay duration, starting target location, and target speed (range: 10-60 deg/s) and direction (inward, outward, upward, downward) were varied randomly to elicit saccades with different vectors (amplitude and directions). Delay trials using stationary targets placed along moving target paths were randomly interleaved with delay trials using moving targets. Eye position was recorded using magnetic search coils and an eye-tracker system for non-human primates and humans, respectively. Preliminary data are similar for both species. Analyses indicate that saccade metrics between stationary and moving targets may be more similar than previously proposed (Guan et al. 2005, Keller et al. 1996). We observed no differences in the duration, peak velocity, average velocity, latency, and saccadic error between amplitude matched saccades used to foveate stationary and moving targets across directions and speeds. The discrepancies between our observations and those previously reported could be a function of task differences. For instance, we used a delay saccade task and most previously reported data were collected using a step saccade task. We also interleaved many more target trajectories than previous studies. These results suggest that the interceptive saccade vector encoded in the programming pathway is transformed in a similar temporal pattern in the brain stem as traditional saccades.

Disclosures: K.J. Mohsenian: None. **B. Steinberg:** None. **A.L. Cecala:** None. **N.J. Gandhi:** None.

Poster

715. Vision and Eye Movements

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Topic: E.01. Eye Movements

Support: Natural Sciences and Engineering Research Council of Canada

Title: Frontoparietal functional connectivity in the common marmoset

Authors: *M. GHAHREMANI[1,2], M. HUTCHISON[4], J. S. GATI[2], K. GILBERT[2], R. S. MENON[1,2], S. EVERLING[1,2,3];
[1]Grad. Program in Neurosci., [2]Robarts Res. Inst., [3]Dept. of Physiol. and Pharmacol., Univ. of Western Ontario, London, ON, Canada; [4]Ctr. for Brain Sci., Harvard Univ., Cambridge, MA

Abstract: Interest in the common marmoset monkeys (Callithrix jacchus) is growing rapidly as it is poised to become the leading candidate transgenic primate model. In contrast to the well-established Old World macaque, little is known about the functional organization of the saccade circuitry in these New World primates. Here, we used resting-state ultra-high-field fMRI data collected from 4 lightly anesthetized male marmosets and 12 anesthetized male macaques to examine and compare the brain's functional organization, with emphasis on the saccade system. Macaque data and marmoset data were obtained using custom-built transmit/receive coils on a 7T and 9.4T MR scanner, respectively. Exploratory independent component analysis (ICA) revealed eight resting state networks in marmosets that greatly overlapped with corresponding macaque and human networks including a distributed frontoparietal network. Seed-region analyses of the superior colliculus (SC), a fundamental node in saccadic eye movement control, showed homologous functionally connected areas in both macaques and marmosets. Visualization of surface rendered functional connectivity maps revealed the strongest bilateral connectivity of the SC in frontal areas 6DC, 6DR, 8C, 8B, 8aV, 8aD, 46D, parietal areas PG, PGM, PO, MIP, LIP, and temporal areas MT, MST, FST of marmosets. Considerable functional connectivity was also observed in visual areas V1, V2, V3, V4 as well as cortical face patch areas ML, MF, AL, AF and the amygdala. Functional connectivity of the marmoset frontal eye fields revealed a similar connectivity pattern including a strong functional connectivity with areas 23a, 23b, 23c, 24m, 29a-c and parietal area PF. Furthermore, areas 8aD, 8aV, PG, TPO, TE2, and TE3 were identified as major hubs based on a region-wise evaluation of betweeness centrality, suggesting that these cortical regions make up the functional core of the marmoset brain. The results support an evolutionarily preserved frontoparietal system and provide a starting point for invasive neurophysiological studies in marmosets.

Disclosures: **M. Ghahremani:** None. **M. Hutchison:** None. **J.S. Gati:** None. **K. Gilbert:** None. **R.S. Menon:** None. **S. Everling:** None.

Poster

715. Vision and Eye Movements

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Program#/Poster#: 715.19/LL1

Topic: E.01. Eye Movements

Support: Canadian Institutes of Health Research

Title: Oculomotor gap effect and reduced saccadic reaction times to faces in the common marmoset

Authors: *K. D. JOHNSTON, S. EVERLING;
Univ. of Western Ontario, London, ON, Canada

Abstract: The common marmoset (Callithrix jacchus) is a New World primate that shows considerable promise as a model animal for neuroscience research. The first primate transgenic lines have been developed in this species, making possible the integration of genetic and neurophysiological tools in primates. Practical advantages, such as a lissencephalic cortex that allows recording with linear and planar electrode arrays, and a clear extant knowledge of the anatomy and physiology of the visual system in this species further support it's value. The oculomotor system is the most thoroughly understood sensorimotor system in the brain, and provides a window into numerous neurodegenerative and neuropsychiatric disorders. To date however, a limited number of studies have evaluated the oculomotor behaviour of the common marmoset. Here, we investigated the performance of the common marmoset on an oculomotor phenomenon well-studied in humans and rhesus macaques, the gap effect. This effect is a decrease in saccadic reaction times (SRTs) observed when the fixation spot is extinguished prior to the appearance of a target in saccade tasks. We trained a common marmoset to make saccades to peripheral white circular targets for liquid reward. In a "step" condition, an initial fixation spot was extinguished simultaneously with the appearance of the target. In a "gap" condition, the fixation spot was extinguished 200ms prior to target appearance. Consistent with previous observations in both humans and macaques, we observed reductions in SRTs in the gap as compared to step condition. In a second series of experiments, we expanded the set of target stimuli in the gap condition to include marmoset faces as well as control stimuli consisting of scrambled faces, to investigate the possibility of an SRT advantage for naturalistic stimuli. Under these conditions, we observed a reduction in SRTs to faces as compared to control stimuli. Taken together, these findings suggest that conserved oculomotor circuits mediate the gap effect in marmosets and Old World primates, and support the use of the common marmoset as a model for investigations of oculomotor neurophysiology. Further, they suggest preferential processing of faces in the marmoset oculomotor system.

Disclosures: K.D. Johnston: None. **S. Everling:** None.

Poster

715. Vision and Eye Movements

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Topic: E.01. Eye Movements

Support: DFG EXC 307

Title: Differential effects of saccadic suppression on single-unit activity and local field potentials in the primate superior colliculus

Authors: *C.-Y. CHEN[1,2], Z. M. HAFED[1];
[1]Werner Reichardt Ctr. For Integrative Neurosci., Tübingen, Germany; [2]Grad. Sch. of Neural and Behavioural Sciences, Intl. Max Planck Res. Sch., Tübingen, Germany

Abstract: Saccadic suppression refers to reduced visual sensitivity around the time of saccades/microsaccades. Neuronally, suppression appears as reduced firing rates to visual stimuli, and behaviorally, this translates into, among others, delayed reaction times (RT's). However, the sources of saccadic suppression remain elusive. Here, we compared saccadic influences on both firing rates (single-unit activity; SUA) and local field potentials (LFP's) in primate superior colliculus (SC). We hypothesized that LFP's, a proxy for population and synaptic activity, can reveal movement-related signals at the time of visual burst SUA suppression, which may help constrain the neural sources of a suppressive saccade-related signal. We recorded from 90 SC sites (1-24 deg eccentricity) in 2 monkeys, collecting both SUA and LFP's. Monkeys fixated a white spot over a gray background. Inside RF's at the recorded sites (assessed from SUA and LFP tuning properties), a stationary, vertical Gabor grating (0.56, 1.11, 2.22, 4.44, or 11.11 cpd) appeared. For SUA, we measured peak visual response 20-150 ms after grating onset. For LFP's, we measured peak transient deflection similar to how we measured peak SUA response, and we also collected transient LFP latency defined as the first time at which the LFP was >2 s.d. away from pre-stimulus baseline. We compared trials when gratings appeared <50 ms after microsaccade end (the saccadic suppression trials) to trials when the gratings appeared without microsaccades (baseline). We chose a post-microsaccade interval to ensure that the eye was already stable at the time of grating onset, just like in the baseline trials. Visual-motor neurons showed the strongest visual response suppression, and the suppression was spatial-frequency-dependent; visual-only neurons had only mild and unselective suppression. In the LFP's, opposite to the SUA's, we observed enhanced visually-evoked transient response, and with facilitated latency (regardless of electrode location). This suggests the presence of a movement-related signal that may mediate SUA visual burst suppression. We confirmed this by identifying microsaccade-related LFP modulations, even in the far periphery, in the absence of any visual stimulus. However, the interaction between this movement-related modulation and the

visually-evoked LFP response was supra-additive because the enhanced LFP response could not be explained by a baseline shift caused by the movement-related modulation. Thus, LFP's show opposite saccadic suppression effects from SUA and reveal a putatively movement-related signal at the time of SUA suppression.

Disclosures: **C. Chen:** None. **Z.M. Hafed:** None.

Poster

715. Vision and Eye Movements

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Topic: E.01. Eye Movements

Support: CAS Hundred Talent Program

Title: Temporal compression of visual perception around microsaccades

Authors: ***M. C. DORRIS**, G. YU, M. YANG, P. YU;
Inst. of Neuroscience, Shanghai Inst. For Biol. Sciences, CAS, Shanghai, China

Abstract: Even during fixation, our eyes are in constant motion. For example, microsaccades are small (typically<1°), rapid eye movements that occur during attempted fixation. Recently researchers have begun to characterize how microsaccades influence visual attention and visual perception. Here we demonstrate that microsaccades can also influence time perception; that is, the perceived temporal interval between transient visual stimuli is compressed around microsaccades. While maintaining central fixation, human subjects reported whether the duration between a pair of successively flashed horizontal bars (test interval) was longer or shorter than the duration between a subsequent pair (probe interval). A total of ~40000 trials were collected from 8 subjects. Trials were separated into those with and without accompanying microsaccades. We found that if spontaneous microsaccades occurred around the time of one of these temporal intervals (test or probe), that temporal interval was judged to be of shorter duration compared to those trials without microsaccades. This temporal compression was consistent across subjects and was not influenced by attributes of microsaccade such as their particular direction or amplitude. Microsaccades exerted their strongest effect when they occurred within 100ms of the temporal interval and significant effects extended for ~250ms both before and after the temporal interval. The magnitude of the temporal compression
was larger if a microsaccade was associated with the later probe interval than if a microsaccade was associated with the earlier test interval. If multiple microsaccades occurred surrounding a temporal interval, this enhanced the temporal compression of that interval. If microsaccades

occurred surrounding both temporal intervals, their temporal compression effects tended to counteract each other. The compression of time surrounding microsaccades resembles the temporal compression associated with voluntary (macro)saccades (Morrone et al., 2005). Considering microsaccades' small and fast nature, this compression effect on visual perception is far-reaching. In the spatial dimension, the flashed visual stimuli were far (~20°) from central fixation. In the temporal dimension, microsaccades exerted an influence for +/- 250ms from their onset. Together these suggest an underlying extra-retinal mechanism rather than a mere retinal impact. Lastly, given the ubiquity of microsaccades (1~2Hz), our results raise questions of how we can maintain accurate time perception in our everyday life and during experimental situations.

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Poster

715. Vision and Eye Movements

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Program#/Poster#: 715.22/LL4

Topic: E.01. Eye Movements

Title: Neural activity and saccadic eye movements involved during letter and object stimuli manipulations

Authors: ***N. Z. AL DAHHAN**[1], D. C. BRIEN[1], J. R. KIRBY[2], D. P. MUNOZ[1];
[1]Queen's Univ. Ctr. for Neurosci. Studies, Kingston, ON, Canada; [2]Queen's Univ. Fac. of Educ., Kingston, ON, Canada

Abstract: Although reading is an important and generative skill, it remains controversial how it develops and how dysfunctions lead to reading difficulties. To further understand the processes that are involved during reading, we combined functional magnetic resonance imaging (fMRI) with eye tracking to investigate the neural substrates and cognitive processes underlying performance during letter and object naming speed (NS) tasks. These tasks, which measure how quickly and accurately participants can name sets of familiar stimuli, provide a simplified example of processes involved during reading and are a precursor and concurrent correlate of accurate and efficient reading.
We recruited 19 healthy young adults (ages 21 - 26 years), and employed a block design consisting of a letter NS task and three variants that were either phonologically and/or visually confusing; and an object NS task with a variant in which the object names rhymed with one another, while subjects' eye movements and articulations were recorded. We examined how these manipulations influenced behavioral performance and whether they resulted in differences

in neural activation.
Behavioral analyses revealed that letter NS manipulations were influenced by visual rather than phonological similarity. When the task was both visually and phonologically similar, participants had significantly longer naming times and fixation durations, and made more frequent saccades and regressions. For the object NS tasks participants' performance was not affected when the names of the objects rhymed with one another.
fMRI results indicated that these NS tasks activated key neural structures that are involved in reading, such as: the ventral visual stream (visual word-form area), supramarginal gyrus (grapheme-phoneme mapping), middle temporal gyrus (semantic access), motor cortex (motor planning), supplementary motor and pre-motor areas (articulation), and anterior cingulate (speech monitoring). Activation in the temporoparietal areas of the reading network varied by difficulty of each letter NS task indicating differential neural processes that are associated with each task. There was also significantly greater bilateral activation in the fusiform gyrus and parahippocampal gyrus during the object NS tasks than the letter NS tasks.
These findings further our understanding of the neural substrate required for reading. As further progress is made in understanding these underlying processes, this could lead to establishing biomarkers to help identify children who may be at-risk for developing reading difficulties in order to provide them with early assessments and effective interventions.

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Poster

715. Vision and Eye Movements

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Topic: E.01. Eye Movements

Support: Belgian Program on Interuniversity Attraction Poles

Actions de Recherche Concertées (French community, Belgium)

European Space Agency (ESA) of the European Union

Title: Integration of past and current visual information during eye movements in Amblyopia

Authors: ***N. DERAVET**[1], D. YÜKSEL[2], J.-J. ORBAN DE XIVRY[4], P. LEFÈVRE[3];
[2]Inst. of Neurosciences ; Ophtalmology department, [3]Inst. of Neurosciences ; ICTEAM, [1]Univ.

catholique de Louvain, Louvain-la-Neuve, Belgium; [4]Dept. of Kinesiology, Movement Control and Neuroplasticity Res. Group, Katholieke Univ. Leuven, Leuven, Belgium

Abstract: Amblyopia is a neuro-developmental disorder characterized by a reduced acuity of one or both eyes that is not related to a structural, correctible deficit. In addition to acuity and contrast sensitivity deficits, Amblyopia affects perception and eye movements. Furthermore, some of these deficits extend to the fellow eye, excluding loss of acuity as their unique cause. One of the proposed hypotheses is that the integration of visual and extraretinal signals (memory, proprioception…) matures differently in amblyopic patients. In this study, we investigate this hypothesis and the integration of visual signals with memory of target motion during eye movements in amblyopic patients.

Fourteen control subjects and nineteen amblyopic patients, their dominant/fellow eye covered, visually pursued a moving red dot (diameter=0.8°) for a variable number of repetitions (1 to 4 trials). Control subjects had normal or corrected to normal vision, while amblyopic patients had visual acuity of 2/20 to 10/20 Snellen, and exhibited either anisometropic (n=5), strabic (n=9) or anisometropic and strabic (n=5) amblyopia. Repetitions of target motion had the same direction (among 6: 0°±20°, 180°±20°) and the same velocity (among 2: 15°/s, 20°/s), which led to the gradual formation of a memory of target motion. We tested the relative weights of visual and memory signals by presenting, at the last repetition, a catch trial that had the same direction but reduced (-5°/s) or increased (+5°/s) velocity.

Catch trials were compared to control trials that had both the same number of preceding trials and the same velocity. Doing so, the only difference between them was prior information about target velocity.

We found that, for all groups, smooth pursuit eye velocity gain during steady-state was biased towards the target velocity of previous trials ($p<0.05$). Furthermore, the amplitude of catch-up saccades was larger when previous target velocity was higher, and reciprocally ($p<0.05$). Interestingly, the magnitude of the effect did not differ significantly between controls and amblyopic patients (interaction of trial type vs subject type: $p>0.5$), despite obvious differences in visual acuity. It therefore appears that Amblyopia does not significantly affect the weighting of the memory of target motion with visual information during eye movements (within 4 trials). Since we considered a diverse group of amblyopic patients, we intend to pursue this study by increasing our pool of patients and refine the results by type of amblyopia.

Disclosures: **N. Deravet:** None. **D. Yüksel:** None. **J. Orban de Xivry:** None. **P. Lefèvre:** None.

Poster

715. Vision and Eye Movements

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 715.24/LL6

Topic: E.01. Eye Movements

Support: NIH Grant EY10217

NIH Grant EY02162

Title: Normal correspondence of tectal maps for saccadic eye movements in strabismus.

Authors: *J. R. ECONOMIDES, D. L. ADAMS, J. C. HORTON;
Beckman Vision Ctr., UCSF, San Francisco, CA

Abstract: The superior colliculus is a major brainstem structure for the production of saccadic eye movements. Electrical stimulation at any given point in the motor map generates saccades of defined amplitude and direction. It is unknown how the saccade map is affected by strabismus. Three macaques were raised with exotropia by performing a medial rectus muscle tenotomy in each eye at age 1 month. Once the animals were mature, their ocular motor behavior was tested. The animals showed no evidence of amblyopia; they were able to make saccades to targets with either eye and alternated fixation freely. To probe the organization of the superior colliculus, microstimulation was applied at multiple sites, with the animals either free-viewing or fixating a target. In two animals, microstimulation drove conjugate saccades. They were similar in both amplitude and direction, but separated by the ocular deviation. In the third animal, saccades from microstimulation were the same in amplitude, but differed in direction for the two eyes by 15° of polar angle. This direction difference was due to a strabismic pattern deviation that also caused a 15° rotation during static fixations and smooth pursuit. Given that smooth pursuit is not thought to be mediated by the superior colliculus, the rotation in relative eye motion was unlikely to be caused by a shift in the tectal saccade map for one eye. These data indicate that the map for saccade generation appears normal in strabismus, with no evidence for anomalous correspondence. However, saccades generated by tectal stimulation may be disconjugate in animals with a pattern deviation, presumably due to altered downstream elements. Single cell recordings made in the course of these stimulation experiments showed normal, binocular sensory responses in the tectum, in contrast with the striking loss of binocularity induced in striate cortex by strabismus.

Disclosures: **J.R. Economides:** None. **D.L. Adams:** None. **J.C. Horton:** None.

Poster

715. Vision and Eye Movements

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 715.25/LL7

Topic: D.08. Visual Sensory-motor Processing

Title: Perceived location of a target flash presented before, during and after smooth pursuit eye movement

Authors: *J. POLA, H. J. WYATT;
Dept. of Biol. and Vision Sci., SUNY Col. Optometry, New York, NY

Abstract: Smooth pursuit eye movements allow one to visually track a target moving against a stationary background field. Of particular interest, the background is perceived as stationary even though its image, as a consequence of the pursuit, moves relative to the retina. One explanation for this perception is that, associated with the motor signal driving smooth pursuit, an extraretinal (exR) signal is generated that serves to cancel out what otherwise would be the appearance of background displacement. A large number of studies have explored this exR signal in a variety of circumstances. However, there have been no studies concerned with the overall temporal features of the exR signal before, during and after smooth pursuit. To investigate the exR signal over time, subjects in the present experiment observed a target flash (10 ms) presented at various times before, during and after smooth pursuit movement. The subjects reported on the location of the flash relative to the location of a fixation target viewed and extinguished shortly before pursuit onset. (The pursuit target moved either to the right or left at 15 deg/sec for 1.2 sec.) By varying target flash location over experimental trials we determined a target flash that appeared to be at the location of the fixation target, i.e., the target point of subjective equality (TPSE), for each time of flash presentation. Generally, the TPSE was not at the actual location of the fixation target: for rightward smooth pursuit, the TPSE was to the left of the fixation target (opposite to pursuit) from about 0.5 sec before pursuit onset until about 0.75 sec after onset. Thereafter, the TPSE was to the right of the fixation target (in the direction of pursuit) during the remainder of pursuit and 2.5 sec of fixation after pursuit. Using the pursuit time course, it was possible to find a *retinal locus* corresponding to each TPSE, i.e., the retinal point of subjective equality (RPSE). In general, the RPSE shifted relative to the retina in a compensatory direction (for retinal image displacement) as a linear function of time. However, the RPSE shift began before pursuit onset, occurred more slowly than the pursuit movement, and reached a final magnitude after the pursuit (during post pursuit fixation) that was smaller than that of the eye movement. In short, the RPSE suggests an exR signal that changes more slowly than pursuit movement with a final signal magnitude that is smaller than that of the movement. The features of the TPSE, RPSE and underlying exR signal are considered in view of the functional dynamics of the smooth pursuit system and the possible influence of target flash visual persistence.

Disclosures: **J. Pola:** None. **H.J. Wyatt:** None.

Poster

715. Vision and Eye Movements

Location: Halls B-H

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Program#/Poster#: 715.26/LL8

Topic: D.08. Visual Sensory-motor Processing

Support: SUNY Eye Institute Grant

SUNY Brain Network of Excellence Award

NIH R01-EY014885

Title: Macaque use of categorical target templates to guide visual search for complex naturalistic stimuli.

Authors: ***B. M. COOPER**[1], H. ADELI[2], G. J. ZELINSKY[2], R. MCPEEK[1];
[1]Biol. Sci., State Univ. of New York Col. of Optometry, New York, NY; [2]Dept. of Psychology, Stoney Brook Univ., Stoney Brook, NY

Abstract: Humans represent objects as higher-level categories. While object category representations have also been demonstrated behaviorally and physiologically in macaques, macaque use of categorical target templates to guide visual search has yet to be demonstrated. Here we investigated target guidance with two search tasks, one in which a target exemplar was previewed (exemplar search) and another in which the target was defined by a category-specific symbol (categorical search). Subjects freely searched for a member of an object category (e.g. teddy bears), among distractors drawn from random non-target categories (e.g. cars, plants, utensils, etc.). Objects were arranged on a circle and displayed at 10 deg. eccentricity from central fixation. Set size was varied over five levels (2-8, evenly spaced). On target-present trials (TP; 50%), the subject was rewarded after making a saccade to the cued target. On target-absent trials (TA; 50%), reward was given after fixation was held at a 'Target Absent Button' in the periphery (18 deg.). Search behavior was also compared to predictions of an image-based model of the superior colliculus (MASC) that generates sequences of saccades as it searches for the same exemplar and categorical targets. We found that the proportion of TP trials in which the target was the first-fixated object was highly above chance and didn't differ between exemplar and categorical search conditions, demonstrating a strong level of target guidance. Similar strong target guidance and set size effects were observed in MASC's fixations. We exploited the fact that thousands of opportunities existed to observe the preferential fixation of distractors, from

which we assembled most-fixated and least-fixated sets. We show that the same distractors that were more/less fixated by the macaque were also more/less fixated by MASC. We interpret these findings as evidence that macaques use categorical features in visual search to guide their overt attention. The fact that these features can be extracted by our model and used to predict fixation behavior may make possible the future prediction of neural activity in oculomotor structures such as the superior colliculus in naturalistic tasks.

Disclosures: **B.M. Cooper:** None. **H. Adeli:** None. **G.J. Zelinsky:** None. **R. McPeek:** None.

Poster

715. Vision and Eye Movements

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Topic: E.01. Eye Movements

Support: CIHR operating grant -MOP 93796

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Title: Population coding in the superior colliculus may contribute to variability in saccadic reaction time

Authors: ***S. DASH**[1], T. R. PEEL[2], B. D. CORNEIL[2];
[1]Physiol. and Pharmacol., Robarts Res. Institute, Western Univ., London, ON, Canada; [2]Physiol. and Pharmacol., Western Univ., London, ON, Canada

Abstract: In primates, the saccadic reaction time (SRT) to look towards a visual target ranges from below 100ms to above 300ms. What is the neural basis of such variability? Previous research on the activity of single neurons has highlighted the importance of stimulus properties (e.g., contrast, number of targets) on the vigor of the initial sensory response, and the impact of top-down signal (e.g., expectancy or reward) on low-frequency preparatory activity prior to stimulus onset. Here, we examine the potential contribution of population coding in the oculomotor system to SRT variability. To do this, we recorded saccade-related neurons (currently > 100) in the primate superior colliculus (SC) while monkeys made visually-guided saccades into the neuron's response field. As expected, across SC neurons the time of peak saccade-related activity (Peaktime) correlated positively with SRT, being delayed for longer-latency saccades. However, across our sample of neurons we observed substantial variability in the strength and slope of this correlation, meaning that the time of peak activity on an individual neuron basis was an insufficient predictor of SRT. To investigate population coding, we sorted

all the trials across all the neurons based on increasing SRT, and divided this dataset into partially overlapping sub populations(13 sub-populations; 20ms bins; 5ms separation between bins; range 120-200ms). Unlike what was found with individual neurons, the average Peaktime across all neurons and across all bins correlated nearly 1:1 with SRT. However, we observed a monotonic increase in the variability of Peaktime with increasing SRT. As a consequence of such desynchronization, population activity at saccade threshold decreased for longer-latency SRTs. Together, our results suggest that the synchrony of saccade-related activity in the SC population impacts SRT; it takes longer for a more desynchronized population to reach saccade threshold. Our results also show that saccade threshold is not invariant in this simple task, instead decreasing for longer SRTs. This observation can be reconciled if the downstream brainstem burst generator acts as a leaky integrator accumulating activity from a population of SC neurons with a short time constant.

Disclosures: **S. Dash:** None. **T.R. Peel:** None. **B.D. Corneil:** None.

Poster

715. Vision and Eye Movements

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Topic: E.01. Eye Movements

Support: R01EY021228

T32NS073553

NSF graduate research fellowship

Title: Perceptually driven gain modulation of neuronal activity in the superior colliculus during urgent choices

Authors: ***C. K. HAUSER**, E. ROGERS, T. R. STANFORD, E. SALINAS;
neurobiology and anatomy, Wake Forest Sch. of Med., Winston Salem, NC

Abstract: The superior colliculus (SC) has been implicated in visual target selection, visuospatial attention, and saccade execution. By and large, neural correlates of these processes have been demonstrated in laboratory tasks that, by design, impose serial order to the perceptual decision and motor planning processes that precede saccade execution. While successful in distinguishing purely sensory-contingent from purely motor-related activity, such tasks obscure the dynamics of the perceptual-motor interactions that one would expect in more natural settings. To examine such interactions and their neural correlates, we recorded from single SC neurons

(visual, visuomotor, and motor) while monkeys performed a novel, urgent two-choice task that requires subjects to form a motor plan in advance of receiving a cue that reveals the identities/locations of target and distracter. Crucially, performance on this task varies from chance to near 100% as a function of processing time (PT) — the amount of time available to view the cue before commitment to a saccadic choice. The resulting psychophysical function ("tachometric" curve), which plots success rate as a function of PT, provides an opportunity to examine the relationship between the evolving perceptual decision process and its neural correlates with unprecedented temporal resolution. As we previously demonstrated for the frontal eye field (FEF), the impact of the perceptual judgment was clearly evident as choices progressed from uninformed to informed; i.e., the rising activity associated with target and distracter exhibited characteristic PT dependencies. In addition, we observed an entirely novel form of gain modulation: for most SC neurons, the maximum firing rate either increased or decreased as a function of PT. This PT-dependent gain modulation was observed in all neuronal subclasses in the SC, regardless of their visuomotor properties. Moreover, its magnitude and time course varied across experimental conditions (easy vs difficult), outcomes (correct vs incorrect), and cell classes. The results provide critical insight about how perceptual information is dynamically translated into motor output.

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Poster

715. Vision and Eye Movements

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Topic: E.01. Eye Movements

Support: NIH Grant R21EY025550 (WZ)

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Title: Passive eye movements induced by electromagnetic force (EMF) in anesthetized rats

Authors: Y. YU[1], L. CHEN[1], D. S. SANDLIN[1], J. HUANG[1], S. WANG[1], J. ALLISON[1], H. ZHU[1], *W. ZHOU[1,2];
[1]Dept. of Otolaryngology and Communicative Sci., [2]Dept Neurobiology& Anatom. Sci., Univ. of Mississippi Med. Ctr., Jackson, MS

Abstract: Many important sensorimotor functions (e.g., visual localization and eye movement control) require accurate information of eye position in the orbit, which can be provided by either an efferent copy of the feedforward oculomotor commands or the feedback signals from the extraocular muscle proprioceptive pathways. Whereas the neural substrates for oculomotor commands have been extensively studied, the central processing of extraocular muscle proprioceptive signals remains to be elucidated. A technical barrier to investigate this issue is a lack of noninvasive approaches to induce well-defined passive eye movements that specifically activate eye muscle proprioceptive pathways. In this study, we describe a noninvasive method to generate passive eye movements in isoflurane-anesthetized rats (N=6). A small magnet (<1mm) was attached to the surface of a rat's eyeball nasal to the cornea without blocking the pupil, and a small iron rod (~5mm diameter) surrounded by an electromagnetic coil was placed close to the magnet (<15 mm). By passing currents with different polarities to the electromagnetic coil, attractive or repulsive electromagnetic force was generated upon the magnet attached to the eye, therefore inducing eye movements. A video-based eye tracker (ISCAN ETL-200) was employed to measure horizontal and vertical positions of the stimulated eye at a speed of 240 frames per second with a spatial resolution of 0.1 deg. The results show that the electromagnetic forces generated by the coil induced well-defined eye movements: directions were dependent on current polarity, and amplitudes (1~15 deg) and peak velocities (100~1000 deg/s) were dependent on current intensity (0.1~0.7A) and pulse duration (20~200ms). We further showed that peak velocities of the EMF-induced eye movements were linearly related to their amplitudes, demonstrating consistent main sequences among rats. Besides the slopes, the main sequences of the EMF-induced eye movements were similar to that of saccades in awake rats and that of eye movements-induced by electrical micro-stimulation of the abducens nucleus in anesthetized rats. These results suggest that the EMF method provides an effective noninvasive approach to induce well-defined passive eye movements for investigating the central processing of eye muscle proprioceptive signals and its role in visual localization and eye movement control.

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Poster

715. Vision and Eye Movements

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Topic: E.01. Eye Movements

Support: ERC Grant AG324070

Title: Abstraction of 2D head-centered positions from tracking a moving visual target: A study in the non-human primate

Authors: *C. BOURRELLY[1,2], R. M. ETOUMBE[1,2], P. CAVANAGH[2,3], L. GOFFART[1]; [1]CNRS Inst. Des Neurosciences De La Timone, Marseille, France; [2]CNRS Lab. Psychologie de la Perception, Paris, France; [3]Dartmouth Col., Dept. of Psychological and Brain Sci., Hanover, NH

Abstract: The ability to navigate in an environment involves notions such as places, distances and directions. Many studies have investigated how the brain represents or encodes these spatial parameters in various navigation tasks. However, very few have tried to explain how these parameters were constructed in naive animals. We developed a simple protocol for studying this problem using the oculomotor system as a probe. In this protocol, monkeys were trained to fixate a central fixation target and to track it when it started to move along a triangular path. The target moved with a constant speed (10°/s) for approximately 5000 ms along one of 4 pseudo-randomly selected paths in the upper, lower, left or right visual fields. The target ended its motion when it returned back to the center of the screen, at which time the monkey received its reward. From a head-centered perspective, the target motion was clockwise. The direction of target motion changed abruptly at the vertices of each triangular path and the oculomotor behavior around these inflection points was an indicator of the monkey's spatial knowledge of the trajectory. Three monkeys were used to study the evolution of their oculomotor tracking during this task. During the first days of practice, the tracking was primarily composed of saccades and the monkey did not anticipate the changes in target motion direction. After a few days of learning, pursuit became more frequent and as the eye approached the inflection points, the velocity of the pursuit eye movements and the amplitude of saccades both decreased. These results indicate that the location of inflections points were encoded, and possibly represented within a spatial map. To further examine this location coding, we tested the tracking behavior in response to an increase in target speed, to an increase in the length of the paths and to a change in motion direction (from clockwise to counterclockwise). During these tests, the ocular tracking revealed the properties of the memory of the inflection points. This new protocol can guide fRMI studies to the brain structures involved in the coding of 2D head-centered spatial memory. Altogether, our work demonstrates the critical role played by the oculomotor system in the spatial mapping of the world.

Disclosures: C. Bourrelly: None. **R.M. Etoumbe:** None. **P. Cavanagh:** None. **L. Goffart:** None.

Poster

716. Visual Sensory-Motor I

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Program#/Poster#: 716.01/MM5

Topic: D.08. Visual Sensory-motor Processing

Support: MIUR

National Health and Medical Research Council Grant APP1082144 (Australia)

FIRB 2013,RBFR132BKP

Title: Temporal stability of reference frames in a 3d reaching task in monkey posterior parietal area V6A.

Authors: K. HADJIDIMITRAKIS[1], R. BREVEGLIERI[2], F. BERTOZZI[2], P. FATTORI[2], *C. GALLETTI[2];
[1]Monash Univ., Melbourne, Australia; [2]Univ. Bologna, Bologna, Italy

Abstract: Neurons in the medial posterior parietal area V6A of macaques have been consistently reported to show spatial modulations during all the phases of an instructed delay reaching task. In addition, during the planning and the execution of hand movements in 3D space performed from different starting hand positions, the vast majority of V6A neurons represent targets' location either in body-centered frame of reference, or in mixed body/hand-centred coordinates, with only occasional units showing pure hand-centered coding (Hadjidimitrakis et al, 2014, Cer. Cortex 24(12):3209-20). We here characterized the frames of reference in earlier epochs of the task, i.e. immediately after target fixation and in the subsequent main part of the delay period and examined whether the reference frames in individual neurons are stable across the task epochs. We recorded the activity of single cells in V6A while two *Macaca Fascicularis* performed in darkness a foveal reaching towards 9 targets located at different depths and in different directions. The reaches could start from two different hand positions, one close to the body and the other far from it, at a different depth. We report no evidence of hand-centred coding also in the earlier phases of the task. Shortly after target fixation and throughout the main part of the delay period, V6A neurons used either body-centred, or mixed body/hand-centred reference frames. Most of the cells showed consistent reference frames across epochs. Interestingly, a population trend for a shift from mixed body/hand-centred frames to 'pure' body-centred coordinates was found as the task progressed. These findings suggest that in V6A, similar to other parietal areas, the reference frames show a limited degree of temporal evolution. The stronger presence of mixed coding at early task stages could reflect the early involvement of V6A in eye-hand coordination, whereas the increase in spatiotopic representations towards movement execution could be related to its role in online movement control.

Disclosures: **K. Hadjidimitrakis:** None. **R. Breveglieri:** None. **F. Bertozzi:** None. **P. Fattori:** None. **C. Galletti:** None.

Poster

716. Visual Sensory-Motor I

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Topic: D.08. Visual Sensory-motor Processing

Support: European Research Council, grant agreement 268970

Title: Using sponstaneous behaviour to interrogate the mouse visual system: a proof of concept

Authors: ***R. STORCHI**, R. A. BEDFORD, F. P. MARTIAL, J. WYNNE, R. J. LUCAS; Univ. of Manchester, Manchester, United Kingdom

Abstract: Mouse spontaneous behaviour is strongly affected by visual cues and by ambient light (irradiance). Their natural tendency to explore new environments and follow simple visual stimuli allowed researchers to use them to measure the acuity of visual perception and to assess the effect of genetic treatments [1-3]. However our understanding of how mouse spontaneous behaviour is driven by vision is still limited to locomotion or pre-specified behaviours. A more thourough comprehension would provide fundamental insights into how the visual system integrates and processes complex natural visual stimuli and how mice make use of it to survive outside a laboratory. Moreover it would also be important to increase the sensitivity of behavioural tests to assess the effects of treatments aimed at restoring vision. In the present work we target two fundamental limitations of current behavioural analyses: low dimensionality and reliance on cumulative measures. Low dimensional movement tracking, typically based on a single body point, dramatically reduces the number of behaviours that can be discriminated. We increase the dimensionality by combining non-invasive body markers and nonlinear dimensionality reduction. Reliance on cumulative measures (e.g. total distance travelled or average locomotion speed) provides a single number summary of complicated dynamics and averages out potentially important events which may last only a small fraction of the time windows considered. We use multivariate change point analysis to segment the behavioural time series and identify an optimal set of distinct behavioural modules. We show that our technique allows us to get quick and precise measurements of mouse irradiance responses and spatial acuity. **References:** [1] Abdeljalil J et al (2005). Vision Res. 45(11):1439-1446. [2] Lagali PS et al (2008). Nat. Neurosci. 11(6):667-675. [3] Cehajic-Kapetanovic J et al (2015). Curr. Biol. 25(16): 2111-2122.

Disclosures: **R. Storchi:** None. **R.A. Bedford:** None. **F.P. Martial:** None. **J. Wynne:** None. **R.J. Lucas:** None.

Poster

716. Visual Sensory-Motor I

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Topic: D.08. Visual Sensory-motor Processing

Support: NIH Intramural Grant

Title: Modulating inter-hemispheric coherence to influence behavior - a TMS-EEG study

Authors: N. THIRUGNANASAMBANDAM, *M. HALLETT;
Human Motor Control Sec, Natl. Inst. of Neurolog. Disorders and Stroke, Bethesda, MD

Abstract: Inter-hemispheric coherence (IHC) is one of the EEG parameters that measures synchronization of oscillations originating from brain regions of different hemispheres and thereby the functional connectivity between them. We know that IHC is critical for tasks such as midline object recognition that involves merging information obtained from the 2 hemispheres. In a previous study, it was observed that midline object recognition is associated with transient increase in IHC over temporo-parietal areas in the alpha frequency range (Mima et al., 2001). Whether this association is causal, remains unknown. In the current study we aimed at determining the causal association of inter-hemispheric alpha coherence to midline object recognition. Several studies have used TMS as an effective tool to entrain intrinsic brain oscillations. We interfered with the IHC by using unilateral or bilateral, synchronous or asynchronous repetitive transcranial magnetic stimulation (rTMS) pulses. Our hypothesis was that asynchronous rTMS would reduce IHC thereby impairing midline object recognition.
We recruited 13 healthy subjects for the study. All subjects first participated in a screening session where the threshold intensity for blocking object recognition and the optimal coil orientation for TMS were determined using single pulse TMS. In the main experiment session, they were asked to perform the object recognition task in four blocks. During the first block, the individual alpha coherence frequency was determined by recording EEG during object recognition. During the second, third and fourth blocks rTMS was delivered in every trial with simultaneous EEG recording. Trains of 7 TMS pulses were administered over left and/or right lateral occipital gyrus at 80% of threshold intensity blocking object discrimination or 60% maximum stimulator output whichever was smaller.
Our primary outcome measure for the object recognition task was d' (d-prime or sensitivity index). Our results have not shown a significant effect of rTMS on object recognition. The EEG

data is currently being analyzed to look for entrainment effects. We think that this absence of effect on behavior could be due to a ceiling effect in task performance, wrong stimulation site or sub-optimal stimulation paradigm. We are currently performing additional experiments to address the above issues.

Disclosures: N. Thirugnanasambandam: None. **M. Hallett:** None.

Poster

716. Visual Sensory-Motor I

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Program#/Poster#: 716.04/MM8

Topic: D.08. Visual Sensory-motor Processing

Title: Direction-selective units in the frog's basal optic root nucleus and its role in gaze stabilization in prey-catching behavior

Authors: *V. A. BASTAKOV[1], O. Y. ORLOV[1], E. I. KISELEVA[2];
[1]Inst. For Info Transmission Problems RAS, Moscow, Russian Federation; [2]A.N. Severtsov Inst. of Ecology and Evolution RAS, Moscow, Russian Federation

Abstract: Electrophysiological responses from 91 direction-selective (DS) units located in the basal optic root (nBOR) and the adjacent dorsomedial area have been recorded extracellularly in the frog (*Rana temporaria* L.). Based on characteristics of the recorded responses, 23 of the units are considered to be retinal ganglion cells' (RGC; axon terminals). The majority of the remaining 68 units were considered to be DS neurons of the nBOR. The results of our study suggest that in the nBOR area there might be four subtypes of the tegmental and retinal DS units that respond selectively to stimuli moving in the dorso-ventral, ventro-dorsal, caudo-rostral and rostro-caudal directions. The receptive field (RF) sizes of the nBOR DS neurons were estimated to be about 30-60°, while those for the retinal units were significantly smaller - just 6-8°. In response to abrupt darkening within the units' RFs, both the nBOR DS neurons and the RGC DS units respond by a weak discharge. Our results indicate that the frog nBOR DS neurons integrate the inputs from the retinal OFF-type DS units over relatively large segments of the visual field.It will discuss the role of retinal projections to the nBOT in gaze stabilization in frog prey-catching behavior.

Disclosures: V.A. Bastakov: A. Employment/Salary (full or part-time): Institute for information transmission problems RAS. **O.Y. Orlov:** A. Employment/Salary (full or part-time): Institute for information transmission problems RAS. **E.I. Kiseleva:** A. Employment/Salary (full or part-time): A.N. Severtsov Institute of Ecology and Evolution RAS.

Poster

716. Visual Sensory-Motor I

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Program#/Poster#: 716.05/MM9

Topic: D.08. Visual Sensory-motor Processing

Title: Behavioral and cognitive changes following lateral posterior thalamic nucleus lesion

Authors: *R. M. CYSNEIROS[1], L. C. AURORA[2], F. A. SCORZA[2];
[1]Univ. Presbiteriana Mackenzie, Sao Paulo, Brazil; [2]Federal Univ. of São Paulo, São Paulo, Brazil

Abstract: Lateral posterior (LP) thalamic nucleus is part of the cortical–basal ganglia–thalamic–cortical network and appears important for various functions involved with spatial processing related to directed attention, including emotional stimuli. We studied cognitive and behavioral changes in male Wistar rats submitted to unilateral electrolytic lesion of the LP at 60 days post-natal. The behavioral tests started 7 days after surgical procedures. In open field, LP group exhibited higher time of immobility ($F[1,18] = 19.07$; $p = 0.0018$) and self-grooming episodes than SHAM group ($F[1,18] = 15.3$; $p = 0.0036$, respectively). In central zone, LP group exhibited reduced locomotor activity ($F[1,18] = 31.6$; $p = 0.0003$) and time spent ($F [1,18] = 27.29$; $p =0.0005$) as compared to SHAM group. In elevated plus maze, for percentage of time spent and number of entries into the open arms significant differences were observed between groups ($F[1,9] = 132.1$; $p < 0.0001$, $F[1,9]= 7.02$; $p <0.001$, respectively), with no difference for total locomotion ($F[1,9] =0.46$; $p = 0.52$). In the social approach phase of the sociability paradigm, for time spent in the compartments with social and non-social stimuli, significant differences were noted between compartments ($F[1,9]= 83.9$; $p = 0.0001$) and effect of interaction between factors ($F[1,9] = 9.96$; $p = 0.0116$). Both groups exhibited preference for the social stimulus, but LP group showed less discrimination as compared to SHAM group ($t = 4.44$; $p<0.01$ e $t = 8.34$; $p<0.001$, respectively). In the social novelty phase, for time spent in the compartments with familiar and unfamiliar conspecific, significant differences were observed between groups ($F[1,9] = 11.76$ $p =0.0075$) and effect of interaction between factors ($F[1,9] = 11.76$ $p =0.0075$). LP group spent more time with familiar animal as compared with social novelty. For social contacts, it was noted effect of interaction between factors ($F [1, 9] =72.73$; $p < 0.0001$). LP group exhibited more social contacts towards to familiar animals as compared to social novelty, and opposite behavior was observed in SHAM group. In operant conditioning box, SHAM group learned to press the right bar in the first session and the LP group completed the task between the second and fourth sessions, ($\chi^2 = 8{,}049$; $p =0.0046$). For reversal learning, SHAM group learned to press the left bar between first and second session and the LP learned between the third and fourth sessions ($\chi^2 = 9{,}747$; $p =0.0018$). The data suggest that the LP lesion leads to anxiety

related behavior, social behavior impairment, stereotypic behavior, deficits in visual conditional associative learning and the cognitive flexibility.

Disclosures: **R.M. Cysneiros:** None. **L.C. Aurora:** None. **F.A. Scorza:** None.

Poster

716. Visual Sensory-Motor I

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

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Topic: D.08. Visual Sensory-motor Processing

Support: Simons Foundation

NIH 1U01NS090562-01

Title: Neural representation of a threat stimulus in the early visual system

Authors: ***K. LEE**, Z. TURAN, M. MEISTER;
Caltech, Pasadena, CA

Abstract: The mouse shows a rapid, innate defensive response to an overhead expanding black disc that mimics an approaching predator: the animal freezes in place or escapes to a shelter. Driven purely by the visual stimulus, this “looming reaction” provides an opportunity to study how sensory information is transformed to motor output by brain circuits.
Where in the visual system is the looming feature first detected and discriminated from innocuous visual events? It has been suggested that the Off-transient alpha ganglion cell in the retina serves this function (Munch et al. 2009), but recent work shows that this cell type still fires promiscuously during many other episodes encountered in mouse natural vision. Thus the looming detector may lie downstream of the retina, perhaps in the superior colliculus (SC), a layered midbrain area important for sensorimotor transformation.
The superficial layer of the SC serves as the gateway for visual input from the retina but how it represents visual stimuli is poorly understood. Here we study the convergence of retinal input onto the SC by transsynaptic retrograde tracing from genetically identified sSC neurons. We then explore the neural representation of visual stimuli by recording from an awake mouse with silicon neural probes that span all layers of the SC. In the superficial SC we report neurons with diverse visual response properties that collectively encode many different stimulus features. In the deeper layers we find certain neurons with a pronounced response to the onset of the black expanding disc, but not to many innocuous stimuli that fail to elicit a looming reaction. We

suggest that the neural computation of a looming threat emerges in the interlaminar circuitry between the superficial and deeper layers of the SC.

Disclosures: **K. Lee:** None. **Z. Turan:** None. **M. Meister:** None.

Poster

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Support: PFV/10/008

Fonds voor Wetenschappelijk onderzoek

ERC Stg-260607

Title: Spatial and temporal extent of single pulse Transcranial Magnetic Stimulation on single-cell activity in alert macaque monkeys.

Authors: ***M. C. ROMERO**, M. DAVARE, P. JANSSEN;
KULeuven, Leuven, Belgium

Abstract: Transcranial Magnetic Stimulation (TMS) is a safe, non-invasive brain stimulation technique that can induce electric currents in the brain. Despite its extensive use both in the clinical practice and cognitive neuroscience, very little is known about the spatial and temporal extent of TMS effects on single neurons. To study the TMS influence on neuronal activity, we performed simultaneous TMS and electrophysiological recordings in parietal area PFG of two macaque monkeys during a visually-guided grasping task (VGG). We measured the changes in neuronal activity in a region located directly under the center of stimulation, and analyzed the spatial (7 mm antero-posterior, 3 mm medio-lateral and 6 mm dorso-ventral) and temporal extent of TMS effects. In the stimulation trials, single TMS pulses were applied either at 60% (low intensity) or at 120% (high intensity) of the motor threshold. With this protocol, we recorded 378 PFG neurons in two animals and quantified the net evoked activity, comparing the average response of all neurons tested with high intensity TMS before (-70 to -30 ms) and after (30 to 70 ms) stimulation to the same intervals in the absence of stimulation. Single pulse TMS caused a short-latency excitation in PFG neurons, which emerged around 20 ms after stimulation, and lasted for approximately 50-80 ms. Surprisingly, this excitatory effect of TMS was significant (t-test post- versus pre-stimulation interval) in only a single recording position in both monkeys. In this center of stimulation, TMS evoked a significant burst of activity in a substantial number of

neurons (t-test, 25/46, 54% in monkey 1; 21/50, 42% in monkey 2). The net TMS-evoked activity averaged 16.7 (monkey 1) and 30.9 (monkey 2) spikes/sec at the center of stimulation. Even in neighboring recording positions (located 1 or 2 mm away from the center of stimulation, both in the anterior-posterior and medio-lateral directions), TMS did not induce a significant change in activity (average evoked activity less than 10 spikes/sec). Finally, behavioral analyses of the VGG task revealed that single-pulse TMS applied over PFG during the reach-to-grasp movement caused a significant increase in the grasping time in both monkeys. Thus, single-pulse TMS over parietal cortex causes a robust, transient burst of activity in single neurons which is remarkably local, providing the first validation of the effect of single-pulse TMS on single neurons during behavior.

Disclosures: **M.C. Romero:** None. **M. Davare:** None. **P. Janssen:** None.

Poster

716. Visual Sensory-Motor I

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Topic: D.08. Visual Sensory-motor Processing

Support: HHMI

Title: The role of the serotonergic system in short-term motor learning in larval zebrafish

Authors: ***T. KAWASHIMA**, C.-T. YANG, B. D. MENSH, M. B. AHRENS;
HHMI Janelia Res. Campus, Ashburn, VA

Abstract: To execute accurate movements, animals must continuously adapt their behavior to changes in their body and the environment. Animals can learn changes in the relationship between their locomotor commands and the resulting displacement of the body, then adjust command strength to achieve a desired locomotion. It is largely unknown which circuits implement this form of motor learning. We use whole-brain neuronal imaging and circuit manipulations in larval zebrafish to perform a functional screen of brain regions involved in short-term motor learning. We find that the serotonergic system, in particular the dorsal raphe nucleus, stores a memory of learned motor drive that builds up during training. We identify the motor and sensory signals that drive the formation of this memory, and demonstrate causal relationships between raphe activity and behavior.

Disclosures: **T. Kawashima:** None. **C. Yang:** None. **B.D. Mensh:** None. **M.B. Ahrens:** None.

Poster

716. Visual Sensory-Motor I

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Support: NIH Grant NINDS-NS078127

Klingenstein Fund

Simons Collaboration on the Global Brain

Title: Probing a sensorimotor transformation in dorsomedial frontal cortex using electrophysiology and optogenetics

Authors: *E. D. REMINGTON[1], E. A. HOSSEINI[1], M. JAZAYERI[1,2];
[1]McGovern Inst. for Brain Res., [2]Dept. of Brain and Cognitive Sci., MIT, Cambridge, MA

Abstract: A central question in neuroscience is how the brain uses sensory inputs to generate appropriate motor outputs. A recent study used a time interval reproduction task in monkeys to address this question (Jazayeri and Shadlen 2015). Results were consistent with a "preplanning" model in which the brain uses an estimate of elapsed time during the measurement of an interval to compute an ongoing motor plan. However, to test the preplanning model more definitively, it is important assess how the same sensory input can be used to plan different motor responses. Therefore, we designed a variant of the time interval reproduction task in which the relationship between the measurement and production intervals varied between two contexts. In the first context, monkeys had to reproduce the sample interval, similar to the previous study. In the second context, monkeys had to produce an interval that was 50% longer than the sample interval. In other words, the two contexts had similar sensory information but required the animal to apply two different gain factors to compute the appropriate motor plan. We recorded activity in dorsomedial frontal cortex (dMFC) of monkeys measuring and producing time intervals in alternating blocks of trials associated with the two behavioral contexts. dMFC responses in the measurement and production epochs were modulated by elapsed time. Consistent with the preplanning model, the gain factor influenced neural responses during the sensory measurement and the magnitude of the effect was time dependent. To gain further insight into the nature of dMFC signals and to test their causal effect on behavior, we perturbed neural activity in dMFC using optogenetics. We transduced dMFC neurons using a virally mediated red-shifted chloride pump (JAWS, Chuong et al. 2014) that enabled us to modulate activity at multiple time points during the task. In our initial tests with brief optical stimulations, we were able to perturb responses of a substantial fraction of neurons (n = 48/221) in the vicinity of the injection sites. However, these effects were largely limited in time to the stimulation intervals, and stimulation

had no significant effect on task performance. We therefore used a stimulation protocol in which we targeted a larger volume of dMFC for longer durations, which enabled us to influence behavior reliably. Ongoing work aims to use this protocol to further examine how activity in the measurement epoch is used to generate the appropriate motor plan in the production epoch.

Disclosures: **E.D. Remington:** None. **E.A. Hosseini:** None. **M. Jazayeri:** None.

Poster

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Wellcome Trust & Royal Society

UCL Excellence Fellowship

ATIP/AVENIR

Title: Anatomy and function of an inter-hemispheric neural circuit in the zebrafish optic tectum

Authors: ***C. GEBHARDT**[1], T. O. AUER[1], K. DUROURE[1], I. H. BIANCO[2], F. DEL BENE[1]; [1]Ctr. de Recherche, Bat. BDD, Equipe Del Bene, Inst. Curie, Paris, France; [2]Dept. Neurosci., Physiol. & Pharmacol., UCL, United Kingdom

Abstract: The optic tectum is the main retino-recipient brain structure in zebrafish and as such each tectal hemisphere receives direct input exclusively from the contralateral retina respectively. Based on observations of the behavior of zebrafish larvae during capturing of prey, like e.g. paramecia, it is likely that visual signals coming from both eyes of the animal have to be integrated e.g. for efficient hunting (Bianco et al. 2011, Bianco et al. 2015) but the neural substrate for binocular integration is as of yet unknown.
We recently identified a zebrafish line that specifically expresses the trans-activator Gal4 in a novel, previously undescribed neuron population in the visual system of the zebrafish. Using transient UAS:GFP-expression for single-cell labeling in this zebrafish line, we find these neurons to be bilateral-symmetrically distributed directly adjacent to the ventral tectum.

Furthermore, they send their axons through and form arbors in both tectal halves and were thus termed intertectal neurons (ITNs). ITNs are therefore good candidates for the potential transfer and/or the integration of visual signals originating from both eyes.
First, we described the anatomy and temporal development of the ITN neurons and their connections, i.e. their axon trajectories and arbors, potential up- and downstream neurons, the development and distribution of the ITN synapses and also their neurotransmitter type.
Second, to physiologically describe a potential intertectal signal transfer, we performed unilateral eye-ablation at 2dpf and subsequently 2photon-Ca-imaging at 5-6dpf. We observed Ca-transients in the tectal hemisphere not receiving any retinal input that were nevertheless time-locked to the presented visual stimuli. Moreover, these transients were co-localized in the tectum with the position of ITN arbors originating from the contralateral ITNs. Furthermore, we established that ITNs themselves respond to a wide range of visual stimuli including looming stimuli and direction selective bars.
Finally, we tried to understand the role of the ITNs during behavior. To this end we unilaterally ablated genetically stained ITNs using 2photon imaging and subsequently examined free-swimming behavior parameters using a high-speed camera in ablated vs. ctrl fish.
In summary, we anatomically describe novel inter-hemispheric neural connections in the zebrafish visual system and aim to establish their role in inter-tectal visual signal transfer as well as their functional role during behavior.

Disclosures: **C. Gebhardt:** None. **T.O. Auer:** None. **K. Duroure:** None. **I.H. Bianco:** None. **F. Del Bene:** None.

Poster

716. Visual Sensory-Motor I

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Support: Impact Studentship, UCL to PZH

HFSP postdoctoral fellowship to NS

Wellcome Trust 095669 to MC

Wellcome Trust 095668 to KDH

Title: Probing and modeling the causal role of mouse cortical areas in visual discrimination

Authors: *P. ZATKA-HAAS, N. A. STEINMETZ, M. CARANDINI, K. D. HARRIS;
Univ. Col. London, London, United Kingdom

Abstract: Perceptual decisions depend on an interaction between physical stimuli and internal variables such as bias and sensitivity. To investigate the causal role of cortical regions in this interaction, we performed optogenetic inactivation in multiple regions of mouse cortex during a visual discrimination task.
Mice were trained on a two-alternative unforced choice task (Burgess et al, *bioRxiv* 2016). After an auditory cue, grating stimuli of variable contrast appeared in the left and right hemifields. Mice were rewarded for turning a steering-wheel to indicate which hemifield contained a stimulus of higher contrast, or for refraining from turning the wheel (a "no-go" choice) if no stimulus was present in either hemifield. We implanted a head post and transparent skull cap in mice expressing ChR2 in Pvalb-positive cortical interneurons and trained them on the task. On a random subset of trials, a 473 nm laser mounted on a motorized manipulandum inactivated a single site, randomly chosen out of 52 sites over the cortex.
We quantified the mouse's behavior and the effects of focal inactivations using a generalized linear model. This model fits the probabilities of choosing left, right, or no-go in terms of parameters representing bias (b_L,b_R) and stimulus sensitivity (s_L,s_R). The model was expanded to incorporate additive effects of optogenetic inactivation on the bias and sensitivity parameters at different cortical sites. The parameter fits thus estimate the effect of inactivation on these perceptual attributes.
We found that inactivation in primary and secondary visual areas, and secondary motor cortex, reduced unilaterally the bias terms b_L and b_R. This effect corresponds to a choice bias away from contralateral stimuli. We also found that inactivation of primary visual cortex reduces the sensitivity to contralateral stimuli, reflected in a decrease in s_L and s_R. For other cortical regions there was little effect.
These results demonstrate that visual cortex and a subregion of secondary motor cortex are required for the mouse to discriminate visual stimuli based on contrast. Inactivation of either region caused a bias, but only visual cortex determined sensitivity. These findings prepare us to investigate these areas in greater detail, to further uncover the causal neural structures that drive visual discrimination.

Disclosures: P. Zatka-Haas: None. **N.A. Steinmetz:** None. **M. Carandini:** None. **K.D. Harris:** None.

Poster

716. Visual Sensory-Motor I

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Leon Levy Foundation

Fight for Sight Foundation

Title: Adaptation of perception of upright during prolonged head tilts is not mediated by changes in torsion

Authors: *J. OTERO-MILLAN[1], A. KHERADMAND[2];
[1]JOHNS HOPKINS UNVIVERSITY, Baltimore, MD; [2]Neurol., Johns Hopkins Univ., Baltimore, MD

Abstract: Torsional eye movements are rotations of the eye around the line of sight and they occur when we tilt our head towards the shoulder. Perception of upright is usually assessed by a psychophysical task known as subjective visual vertical (SVV) which quantifies the orientation of a visual line that a subject perceives as aligned with the direction of gravity. Torsional eye movements change the orientation of the visual stimulus on the retina thus playing a critical role in our perception of upright. Previous studies have shown that both torsion and SVV can drift over time during prolonged head tilts. Here we directly compared the amount of drift of torsion and drift of SVV during continuous recordings of 15 minutes. We used a novel video tracking method to measure torsion and an adaptive paradigm to continuously measure SVV. Both SVV and torsion were also measured after subjects were brought back to upright position to study any aftereffect caused by the prolong head tilt. We found that torsion always drifted towards zero (the eye position during upright) by an average of 0.8 degrees and between 0 and 3 degrees depending on the subject. SVV, on the other hand, was much more variable across subjects with a drift ranging from 0 to 10 degrees and an average of 5 degrees towards the direction of the head tilt. After the head was brought back to upright position, there was a significant SVV aftereffect of 4 degrees in the same direction as the drift. There was a trend in the aftereffect in torsion but was not significant and only 0.2 degrees on average. The amount of drift in torsion and SVV was not correlated across subjects, nor it was the aftereffect. That is, subjects with larger torsional drifts or aftereffects did not necessarily have larger SVV drifts or aftereffects. Our results show that the drift in upright perception during head tilt and the aftereffect are not due to changes in torsional position of the eyes.

Disclosures: J. Otero-Millan: None. **A. Kheradmand:** None.

Poster

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Support: DFG IRTG 1901

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CIHR (Canada)

Title: Anodal tDCS stimualtion over mIPS improves online control in reaching

Authors: *A. GOETTKER[1], S. XU[2], K. FIEHLER[1], G. BLOHM[2];
[1]Justus-Liebig-University, Giessen, Germany; [2]Ctr. of Neurosci. Studies, Queen's Univ., Kingston, ON, Canada

Abstract: Goal-directed reaches can become inaccurate due to multiple reasons, e.g. sensory noise or changes in the environment. Therefore, the human brain uses an online-control system to correct for movement errors and lead the hand precisely to a target. The posterior parietal cortex (PPC) seems to be crucially involved in online corrections during reaching. When transcranial magnetic stimulation is applied to the PPC, no corrections to sudden changes in target position occur; similar to patients with bilateral lesions in the PPC (Desmurget et al., 1999; Grea et al., 2002).

We wanted to investigate the role of the middle intraparietal sulcus (mIPS) for online corrections during reaching. By using high-definition transcranial direct current stimulation (HD-tDCS) we set out to alter the activity in the mIPS and examined the behavioral effects due to anodal or cathodal stimulation. Subjects took part in the experiment on two days, one with anodal and one with cathodal stimulation. The experiment was divided into 3 sessions: Baseline, Stimulation, Post-Stimulation. Each session included 4 blocks. Each block consisted of 80 trials, 20 each to a left or a right located target and 40 to a central one. On half of the trials to the central target, the target was jumped at hand movement onset to the left or right target location. We measured the curvature of movements to jumping targets and the latency of online correction for hand and eye movements.

The polarity of stimulation significantly changed the curvature of the online correction movements, across sessions. Reaching movements were less curved for anodal stimulation during stimulation and post-stimulation, reflecting better online corrections; they did not change

with cathodal stimulation. In addition, we quantified the influence of stimulation on the online correction latencies for hand and eye movements as the difference between the baseline and each of the upcoming sessions and compared the effects for the polarities. We found that the size and direction of these effects were highly correlated between the hand and eye movements.
In summary, anodal stimulation, which presumably increased mIPS excitability, led to smoother online correction movements. Our results provide further evidence for a crucial role of the mIPS and PPC in general in online correction for reaching. Furthermore, the correlation between the effect of stimulation on the online correction latency of hand and eye movements suggests a common mechanism for both effector systems. This mechanism may be associated with the updating of target locations (Gaveau et al, 2008) or the coupling between effectors during eye-hand coordination (Bowman et al., 2009).

Disclosures: **A. Goettker:** None. **S. Xu:** None. **K. Fiehler:** None. **G. Blohm:** None.

Poster

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Support: Grants-in-Aid for Scientific Research on Innovative areas (15H01576)

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Title: Facilitation of visual-motor response by increasing alpha phase synchronization between visual and motor areas by tACS / tDCS

Authors: *M. KAWASAKI[1,2], Y. HENMI[1];
[1]Univ. of Tsukuba, Ibaraki, Japan; [2]RIKEN Brain Sci. Inst., Saitama, Japan

Abstract: Numerous electroencephalograph (EEG) evidence indicates the important role of sensory-motor phase synchronization in integrating several functions on achievement of stimulus related response. However, it is not well known about the causal relationship, namely, whether or not the observed EEG phase synchronization really induces the behaviors. Using transcranial alternative / direct current stimulation (tACS / tDCS) method to manipulate the effective connectivity in oscillatory sensory-motor networks, we estimate the modulation of the EEG synchronization and that of the sensory-motor response time. First, we confirmed the beta (about 20Hz) and alpha (about 10Hz) phase synchronizations between the visual and motor areas, which is consistent with previous findings. Second, the beta and alpha phase synchronization increased

after the 20 Hz and 10Hz tACS was applied to the visual and motor areas, respectively. Interestingly, the response time after the 10Hz tACS was faster than before the tACS, and the facilitated performance was significantly correlated with the enhancements of alpha but not beta phase synchronization. Finally, the electronic current flows from the input visual area to the output motor area (i.e. in case of the cathodal tDCS to the motor area and the anodal tDCS to the visual area) facilitated the alpha phase synchronization and the response time. These results strongly indicate that the alpha phase synchronization is crucial in mediating effective connectivity among oscillatory network and in facilitating the sensory-motor reaction.

Disclosures: M. Kawasaki: None. **Y. Henmi:** None.

Poster

716. Visual Sensory-Motor I

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Topic: D.08. Visual Sensory-motor Processing

Support: KAKENHI 25119002 15K16009

Title: Transient representation of pre- and post-saccadic information in motion areas MT and MST: a possible neural basis of chronostasis

Authors: *H. KUMANO[1,2], S. KITAZAWA[1,2,3];
[1]Grad. Sch. of Frontier Biosci., Osaka Univ., Osaka, Japan; [2]Ctr. for Information and Neural Networks (CiNet), Osaka, Japan; [3]Dept. of Brain Physiol., Grad. Sch. of Medicine, Osaka Univ., Osaka, Japan

Abstract: During each saccade, visual information is largely lost due to a retinal slip, yet we perceive the visual world as continuous in time. This means that the gap duration is filled with some information. A well-known temporal illusion, chronostasis, suggests that the brain fills the gap duration with post-saccadic visual information (Yarrow et al., 2001). However, this temporal filling-in does not occur when visual information is altered during the saccade. This led us to hypothesize that the brain preserves pre-saccadic visual information until after the saccade for comparison so as to determine whether to fill in the gap or not. In this study, we aimed to test the hypothesis by studying neural responses in motion areas MT and MST around the time of saccades. We recorded extracellular activity of single neurons in areas MT and MST, while two macaque monkeys performed a visually guided saccade task. In each trial, monkeys made a 10-degree saccade toward a visual target that appeared on a tangent display in one of four directions (up, down, left, and right). The display (120 x 90 degrees) was divided into a six-by-nine or five-

by-eight grid of subfields. Within each subfield, a random-dot motion was presented in one of four directions at random: a preferred direction, an anti-preferred direction, and two orthogonal directions. The direction within each subfield was updated every 50 ms. We quantified information about stimulus direction (two bits at a maximum) encoded by spikes of each neuron during a 10-ms time window, which was moved along the peri-saccadic period from -300 ms (before) to +300 ms after the saccade. Neural discharges of MT and MST neurons generally encoded information on motion with a fixed latency of around 50 ms. However, spikes lost any information during an early post-saccadic period from +30 to +70 ms, then at around +80 ms they transiently encoded pre-saccadic information at -80 ms in addition to the default information at +30 ms with the fixed delay. The results clearly show that MT and MST neurons represent visual information just before and just after saccades during the transient post-saccadic period. We infer that the pre- and post-saccadic information during the short post-saccadic period is used for comparison to determine whether to fill in the temporal gap.

Disclosures: H. Kumano: None. **S. Kitazawa:** None.

Poster

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Support: HHMI

Title: Distributed neural prediction of prey motion in amphibians

Authors: *W. R. MOWREY, A. LEONARDO;
Janelia Farm Res. Campus, Ashburn, VA

Abstract: Prediction is a fundamental task of animal nervous systems, but the computations that underlie prediction are largely unknown. Recent work has shown that amphibian tongue projections compensate for phototransduction and head movement delays by extrapolating prey motion (Borghuis and Leonardo, 2015). One hypothesis is that this extrapolation occurs in the retina, as activity in amphibian fast-OFF retinal ganglion cells has been shown to predict object motion (Berry *et al.*, 2013). A tracking estimate to guide the tongue could be extracted from fast-OFF population activity by a population vector average (PVA); if so, tongue projections will have characteristic prey size- and speed-dependent errors arising from fast-OFF cell population dynamics (Leonardo and Meister, 2013). We tested this by presenting toads (*Anaxyrus terrestris*) with artificial prey and recording prey-capture kinematics with high-speed video. Consistent with

fast-OFF/PVA tracking, we find that tongue projections are systematically biased ahead-of-center for prey elongated in the direction of motion. We measured the total visuomotor delay that is compensated by neural prediction by presenting toads with prey that unpredictably reversed direction. The reaction to these reversals shows that prey-capture is not ballistic, as often assumed, but under continuous control from delayed visual signals. Under photopic conditions this reduces the total visuomotor delay by half as compared to ballistic behavior, simplifying prediction. However, toads often hunt under low-light conditions where visual latency can increase dramatically, leading us to ask whether these animals used specific knowledge of the visuomotor delay to predict the prey's future position. We find that dark-adapted animals fail to compensate for increased retinal latency, suggesting that animals can only approximate the system delay. Finally, we tested the ability of toads to use prey velocity information to predict the prey's future position. In contrast to the speed-dependence of the fast-OFF/PVA model, we find that tongue projection accuracy is unchanged over a 4-fold range of prey speeds. Thus, it is likely that additional predictive computation in the brain uses prey velocity information to correct for speed-dependence in retinal tracking. Together, our work shows that distributed neural prediction corrects for prey motion during a visuomotor delay that is minimized by continuous visual control. This prediction accurately corrects for a wide-range of prey velocities, but not for variations in the delay itself.

Disclosures: W.R. Mowrey: None. **A. Leonardo:** None.

Poster

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DFG WI 4046/1-1

DFG KA 3726/2-1

Title: Contribution of dorsal pulvinar to visuomotor behavior and spatial decision-making

Authors: *I. KAGAN[1], A. U. DOMINGUEZ-VARGAS[1], L. SCHNEIDER[1], L. GIBSON[1], M. WILKE[1,2];
[1]German Primate Ctr., Goettingen, Germany; [2]Dept. of Cognitive Neurol., Univ. Med. Goettingen, Goettingen, Germany

Abstract: Evolutionary history and anatomical connectivity of the thalamic dorsal pulvinar suggest its involvement in visuomotor processing and coordination of eye and hand movements. However, since classical visuomotor tasks rarely have been employed in studies of the pulvinar, little is known about its function in goal-directed behaviors. We investigated spatial and effector specificity of neuronal responses in the dorsal pulvinar in two rhesus monkeys performing visually-guided, delayed/memory saccades and free-gaze/eye-hand dissociated delay reach tasks to instructed and chosen locations. Effective connectivity and effects on spatial tuning in interconnected cortical areas of a given pulvinar site were probed by a combination of electrical microstimulation and fMRI. Pulvinar microstimulation potentiated contraversive BOLD signal representations in frontoparietal and temporal cortices. Correspondingly, it led to spatially-specific reaction time and saccade choice changes consistent with overall contraversive "drive", but contingent upon the timing of stimulation and behavioral state imposed by the task. In saccade and reach tasks, dorsal pulvinar neurons exhibited diverse patterns of spatial tuning in cue, delay/memory, pre-, peri- and post-movement epochs; the tuning, as well as the sign of effect (facilitation or suppression) often differed across task epochs. Notably, many cells were modulated by the gaze position and exhibited spatial tuning only after the saccade execution. In saccade tasks across the sample (N = 404), we found no or weak contralateral spatial tuning for either visual cues or oculomotor responses. Thus, no systematic visual or oculomotor map was derived. Some cells were active during fixation but paused during saccades, similar to cells in the superior colliculus. Neurons investigated with reach tasks (N = 110) often showed left/right hand specificity and/or interaction between space and hand variables. Furthermore, visual and saccade-related responses exhibited hand-specific modulations even when a hand was positioned fixedly on the screen throughout entire trial, implying postural interactions. Taken together, our results suggest that hemifield-specific effects of pulvinar inactivation and stimulation are predominately due to its influence on contralaterally-tuned cortical and subcortical regions. The modulation of pulvinar responses by gaze and hand contingencies supports its contribution to coordination of visually-guided actions and transformations between spatial reference frames.

Disclosures: I. Kagan: None. **A.U. Dominguez-Vargas:** None. **L. Schneider:** None. **L. Gibson:** None. **M. Wilke:** None.

Poster

716. Visual Sensory-Motor I

Location: Halls B-H

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Topic: D.08. Visual Sensory-motor Processing

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Wenner-Gren Foundation fellowship (PRM)

Title: A qualitative analysis of the thalamo-cortical and cortico-cortical areal connectivity of the posterior parietal cortex in the Ferret (Mustela putorius).

Authors: *L.-A. DELL[1,2], G. M. INNOCENTI[3,4], C. C. HILGETAG[1], P. R. MANGER[2]; [1]Inst. for Computat. Neurosci., Univ. Med. Ctr. Eppendorf, Hamburg, Germany; [2]Dept. of Anatom. Sci., Univ. of the Witwatersrand, Johannesburg, South Africa; [3]Dept. of Neurosci., Karolinska Inst., Stockholm, Sweden; [4]Brain and Mind Inst., École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland

Abstract: The ferret is an important model for studying brain connectivity due to its elaborate behaviour and well studied physiology. Studies have mapped the cortico-cortical connectivity of the extrastriate cortex and the auditory cortex in the ferret, but none have examined the posterior parietal cortex.
Biotinylated dextran amine (BDA) was injected into the medial and lateral rostral (PPr) and caudal divisions (PPc) of the posterior parietal cortex of the ferret to visualize anterograde and retrograde connectivity of these areas as well as afferent and efferent connections with the dorsal thalamus.
The PPr and PPc in the ipsilateral hemisphere displayed very dense retrograde and anterograde connectivity within these regions as well as the closely surrounding visual regions. The retrograde connectivity tapers off towards the more distal regions while the anterograde connectivity is observed as numerous dispersed clusters of low to moderate terminal networks throughout the cortex. The retrograde and anterograde connectivity of the PPr shows a larger propagation towards the somatosensory and motor cortices than the PPc, which is expected as the PPr receives visual and somatosensory input. The anterograde connectivity of both the PPr and PPc within the contralateral hemisphere displayed the similar extend of weak to moderate 'patchiness' as seen in the ipsilateral hemisphere. Contralateral retrograde connectivity was observed in both the PPr and PPc but the density of these connections were less intense than that observed in the ipsilateral hemisphere and the distribution less dispersed. Furthermore, the distribution of the retrograde connectivity of PPr was confined to the primary motor and somatosensory cortices as well as the posterior parietal cortex whereas that of the PPc extended to the extrastriate cortex. The PPc displayed a more dispersed degree of cortical afferents to the dorsal thalamus than the PPr, however both cortices displayed efferent projections to the thalamus that appeared as bands of moderately dense terminal networks within the lateral posterior, ventral anterior nuclei and pulvinar nuclei. These bands were each enclosed by a halo of weak to moderately dense terminal networks.
Further connectivity studies are necessary to add to the existing cortical 'ferretome' and thus aid modeling of brain connectivity across species.

Disclosures: **L. Dell:** None. **G.M. Innocenti:** None. **C.C. Hilgetag:** None. **P.R. Manger:** None.

Poster

716. Visual Sensory-Motor I

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Topic: D.08. Visual Sensory-motor Processing

Support: NSERC

Title: Transient inhibition of the cerebellum modulates processing of somatosensory input

Authors: *D. ANDREW, R. J. IBEY, W. R. STAINES;
Univ. of Waterloo, Waterloo, ON, Canada

Abstract: Sensorimotor integration (SMI) enables effective responses to sensory stimuli via performance of appropriate motor actions. Cortical contributions to SMI are well established, however subcortical inputs are often neglected or yield inconsistent activity patterns. The cerebellum is emerging as a principle candidate for evaluation of incoming stimuli prior to relaying this information to the somatosensory cortex. Cortical activity associated with stimuli evaluation can be measured using the mismatch negativity (MMN), a response reflective of pre-attentive detection of changes elicited in the 120-180 ms latency range in response to deviant stimuli interspersed amongst frequent stimuli. Typically this is observed fronto-temporally in response to auditory stimuli. Investigation of this response to somatosensory stimuli (S-MMN) has identified abnormalities in the MMN in unilateral cerebellar lesion patients. While this provides a unique population from which to measure responses in affected and unaffected hemispheres, lesion variability may affect results. Therefore, to validate the presence of cerebellar input in the earliest moments of sensory processing, the MMN to somatosensory stimuli was assessed pre and post transient inhibition of cerebellar output. As a secondary outcome of interest, the N24, an early latency peak hypothesized to have cerebellar inputs, was also measured. To do this, continuous theta burst stimulation (cTBS) was performed on the lateral cerebellum ipsilateral to the dominant hand. Participants received frequent and deviant stimulations to the median nerve and on the 5^{th} digit respectively in an oddball paradigm delivered pre and post cTBS. Post blocks were delivered at 3 time points following cTBS (Post 1,2,3 - 0,10,20 min). Scalp responses were averaged and measured using a 64 channel EEG cap. Preliminary results demonstrate a large negativity in response to deviant stimulation pre cTBS in the 120-180 ms latency range, this appears parieto-occipitally. Following application of cTBS, there is a blunting of the MMN such that responses to frequent and deviant stimuli appear similar; this effect is maximal in the Post 3 block. The N24 demonstrates an amplitude decrease apparent during Post 2 and Post 3 time points. Present data indicate that cerebellar processing is crucial for pre-attentive detection of changes in incoming sensory information; evidence of cerebellar input in early latency peaks is also provided. Validating the presence of cerebellar

input for early sensory processing in a healthy population can provide insights into investigating cerebellar abnormalities in aging and disorders with behavioural anomalies.

Disclosures: D. Andrew: None. **R.J. Ibey:** None. **W.R. Staines:** None.

Poster

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Title: Sensorimotor circuit function in orientation and aversion - a common vertebrate plan

Authors: *A. A. KARDAMAKIS, T. WIBBLE, D. SUZUKI, J. PEREZ-FERNANDEZ, S. GRILLNER;
Karolinska Inst., Stockholm, Sweden

Abstract: Sensory-based decisions for redirecting gaze involve the interaction of subcortical and cortical circuits. The optic tectum (superior colliculus in mammals) is central for multisensory integration and motor control of gaze movements (both orienting and aversive). The interaction between multisensory afferent inputs, the basal ganglia and cortex with local tectal circuits play a determinant role in guiding gaze - the synaptic integration of which *remains unknown* on the cellular, synaptic and circuit level.
We have previously shown using the lamprey that the tectal GABAergic system is necessary for generating visual stimulus selection through a process of local excitation and global inhibition for the direct regulation of activity in brainstem projecting neurons (Kardamakis et al., 2015). We found that retinotopic recruitment of GABAergic neurons across the tectum is integrated directly by tectal output cells. Recently, we have also shown that two senses (visual and electrosensory) converge onto the same output neurons with monosynaptic excitatory connections, and that the evoked synaptic currents from the two inputs summate thus potentiating each other when they are aligned in space and time (Kardamakis et al., *review*). Our overarching goal is to map the set of stimuli and afferent synaptic inputs arising from the basal ganglia output nuclei and other forebrain inputs including the pallium (mammalian

homologue of cortex) that selectively activate either contralateral or ipsilateral tectal-brainstem output channel responsible for orienting and aversive gaze movements.
Here, we have developed an isolated preparation that maintains eyes, brain and spinal cord intact enabling us to simultaneously monitor neural responses from the optic nerve, optic tectum, extraocular muscles and ventral roots, while delivering visual stimuli ranging from dots, bars and looming in a computer-controlled environment.
We recorded single units from motoneurons and bursts of ventral root activity in response to dark looming stimuli in the upper visual field in the rostral spinal segments - reflecting episodic fictive head movements that are aversive (*n = 6*). When smaller stimuli are used, phase-tonic activity was recorded unilaterally indicative of an orienting gave movement (*n = 3*). Neural correlates of these gaze reorientation commands were identified by monitoring optic nerve, deep layer tectal and reticulospinal activity during visual stimulation, enabling us to apply pharmacological perturbations to forebrain/midbrain structures including striatum, pallium and pretectum to determine their involvement in shaping this sensorimotor decision-making process.

Disclosures: **A.A. Kardamakis:** None. **T. Wibble:** None. **D. Suzuki:** None. **J. Perez-Fernandez:** None. **S. Grillner:** None.

Poster

717. Eye Movements II

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NIH Grant EY019266

Research to Prevent Blindness

Title: Near and far response cell activity associated with combined saccade-vergence eye movements

Authors: *A. PALLUS[1], M. M. G. WALTON[2], M. J. MUSTARI[2,1];
[1]Ophthalmology, Univ. of Washington, Seattle, WA; [2]Washington Natl. Primate Res. Ctr., Seattle, WA

Abstract: Accurate control of eye movements is critical for humans and other primates who rely on vision to survive. Saccades are conjugate when they occur between equidistant targets, while vergence eye movements rotate the eyes in opposite directions to effect changes in the depth of fixation. This is essential for accurate binocular vision. Vergence eye movements are typically slow, but during saccades between targets at different depths, the vergence velocity is enhanced compared with similar amplitude movements occurring in the absence of saccades.
It is currently unknown whether the observed enhancement behavior is due to an interaction between the neural mechanisms responsible for saccade and vergence movements. Previous studies have identified neurons that may encode vergence velocity, but the activity of these cells has not been described during saccade-vergence movements, so it is unclear whether they encode the enhanced vergence velocity observed during saccades. In the nucleus reticularis tegmenti pontis (NRTP), neurons have been identified with activity related to saccadic eye movement, static vergence angle as well as vergence velocity, suggesting the region may be well positioned to encode saccade-vergence interactions.
In this study, we investigated the activity of individual neurons in the NRTP while monkeys made combined saccade-vergence eye movements to plus-shaped LED targets displayed at varying depths. Our experimental setup allowed for horizontal, vertical and oblique saccades with or without changes in depth. We recorded the activity 29 near and six far response cells in the NRTP of a rhesus macaque performing this task. Of these, 30/35 also showed a burst of activity during saccades with vergence changing in the preferred direction (29 convergent and one divergent), while 31/35 show a pause during saccades that changed vergence in the opposite direction. Most cells showed both pauses and bursts, with a minority only pausing (5/35) or only bursting (4/35).
We further compare these findings with previous recordings from our lab of 27 near and eight far response cells in the supra-oculomotor area (SOA). Only 5/33 neurons in SOA displayed a burst associated with saccades with on-direction intrasaccadic vergence but 26/33 showed a significant reduction in firing rate (below the post-movement baseline) associated with saccades with off-direction intrasaccadic vergence. These data indicate that the NRTP is more sensitive to intrasaccadic vergence velocity than the SOA, while static vergence angle is more strongly represented in the SOA.

Disclosures: **A. Pallus:** None. **M.M.G. Walton:** None. **M.J. Mustari:** None.

Poster

717. Eye Movements II

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Topic: E.01. Eye Movements

Support: NSF Grant 1446377

Title: Mapping the polar angle representation of saccades in human superior colliculus

Authors: *R. R. SAVJANI, E. HALFEN, D. RESS;
Neurosci., Baylor Col. of Med., Houston, TX

Abstract: Purpose: The superior colliculus (SC) is a layered midbrain structure involved in directing eye movements and coordinating visual attention. Electrical stimulation and neuronal recordings in the intermediate layers of monkey SC has revealed a retinotopically organized saccadic eye-movement map. However, the polar angle representation of saccades in ***human*** SC has not been well studied. We used high-resolution fMRI to map the representation of eye movements in human SC. **Methods:** We used a phase-encoding approach similar to previous human studies of saccadic mapping. However, these studies operated with a very low duty cycle (1 saccade every 5 s) and reverse saccades made immediately after forward saccades. We designed a novel paradigm in which subjects performed multiple forward saccades, then returned to the opposite visual field using guided smooth pursuit. Subjects made series of saccades either to left or right (activating primarily the contralateral SC) while we cyclically varied the vertical component of the cue to correspond to saccades in the lower, horizontal, and upper visual field. Attention was engaged via a target discrimination task at the end-point of every saccade and along smooth pursuit. Eight quasi-axial slices covering SC were imaged on a Siemens 3T Trio (2.4 sec/volume, 1.2-mm voxels). Each run consisted of 9, 28.8-s-duration cyclic repetitions of the 3 saccade directions; imaging sessions included 14—16 runs which were subsequently averaged. Sinusoids were fit to the data, yielding phase data that related the fMRI response to saccade angle. Phase encoding with moving-dot stimuli measured the polar-angle representation of the visual field in SC. **Results**: The expected lateral-to-medial phase progression was observed in all subjects (Fig). Also, we found the saccadic maps lie deeper in the SC (intermediate layers) and are in alignment with the more superficial visual-field retinotopy. **Conclusion**: Our techniques in psychophysics and imaging allow us to relate findings in non-human primates to human SC, strengthening our understanding of subcortical vision.

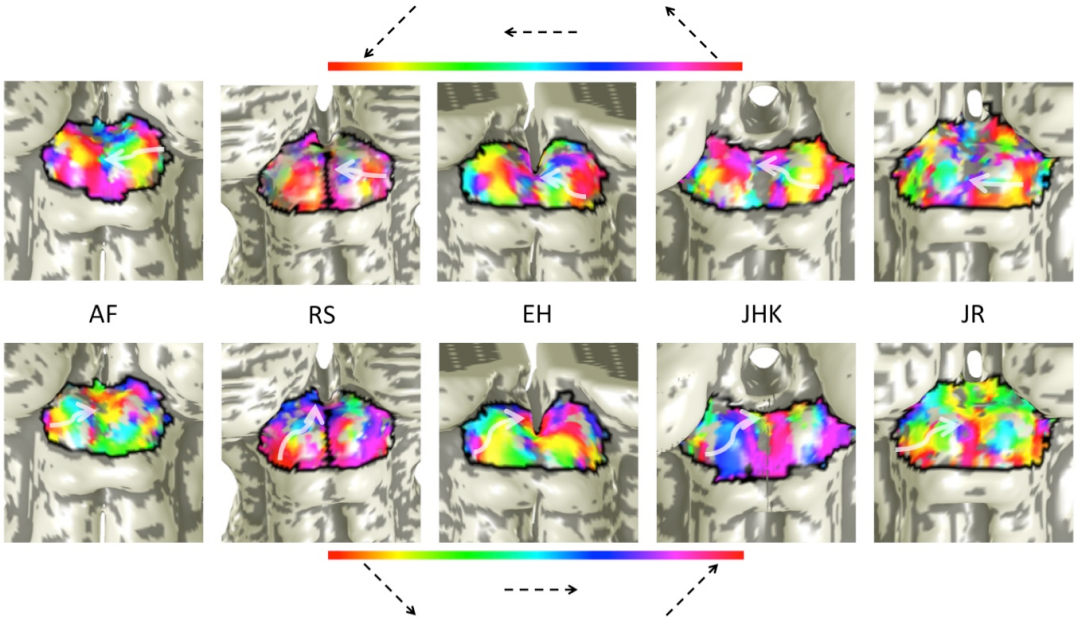

Figure: Data from 5 subjects show eye movements along the inferior-superior visual field axis are mapped along the medial-lateral axis in both SC, consistent with previous studies in alert monkeys.

Disclosures: **R.R. Savjani:** None. **E. Halfen:** None. **D. Ress:** None.

Poster

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Research to Prevent Blindness

Title: Unique compartmental role of inferior rectus (IR) muscle in compensation of vertical strabismus

Authors: ***J. L. DEMER**[1], R. A. CLARK[2];
[1]Jules Stein Eye Inst., [2]Ophthalmology, UCLA, Los Angeles, CA

Abstract: Latent vertical eye position imbalances are physiologically compensated by vertical fusional vergence to prevent diplopia. In some people, such imbalances develop early in life and increase gradually so that enhanced vertical fusional vergence can intermittently control vertical

strabismus. In 4 subjects with intermittent hypertropia (HT) averaging 5.3°, we asked if differential compartmental contractility in cyclovertical extraocular muscles (EOMs) underlies vergence mechanisms that control intermittent strabismus. Surface coil magnetic resonance imaging (MRI) was repeated for each orbit in fusing and deviated conditions, with one eye always aligned to distinguish fusion from monocular fixation of the same target. Contractility, indicated by change in posterior partial volume (PPV), was analyzed by automated algorithm in the medial (equatorial insertion, torsion) and lateral (posterior insertion, vertical) superior oblique (SO) compartments, the superior vs. inferior horizontal rectus compartments, and medial and lateral IR compartments. MRI confirmed no significant vertical or horizontal eye position differences between fusion and monocular fixation. Infraduction of the higher eye to monocular central gaze was associated with 6.2±1.0% (SEM) whole IR PPV increase, with further 3.3±5.2% increase from monocular central gaze to central gaze fusion, in both cases with similar behavior in medial & lateral compartments. Supraduction of the lower eye during monocular switch to fixation was accomplished by 8.3±1.7% PPV decrease in whole IR and 5.6±6.1% increase in whole superior rectus, in both cases with similarly in medial & lateral compartments. The SO behaved similarly with 9.6±5.9% PPV decrease, again similar in both compartments. In contrast, shift from monocular viewing to fusion without position change was associated in the lower eye with 10.1±3.7% PPV decrease in the lateral IR, but 3.8±4.8% increase in the medial IR compartment (P=0.008). No other rectus or the SO exhibited significant whole or differential compartmental contractility during change from monocular fixation to fusion. The lateral IR compartment is innervated by a second motor nerve branch supplementing main IR innervation. Supplemental innervation of the lateral IR may be the basis for unique contribution of the lateral IR to vergence compensation of intermittent HT. Differing contractility of the lateral IR during fusion vs. monocular fixation despite identical eye position confirms absence of a final common pathway in the ocular motor system.

Disclosures: **J.L. Demer:** None. **R.A. Clark:** None.

Poster

717. Eye Movements II

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NSERC RGPIN 341534-07

CIHR Mop-9222

Title: New insights on receptive field remapping with evidence from primate area V4

Authors: *S. NEUPANE, D. GUITTON, C. C. PACK;
Montreal Neurolog. Inst., Montreal, QC, Canada

Abstract: Neural representations of visual space are classically thought to be determined entirely by the pattern of retinal inputs. From this standpoint it is surprising that receptive fields in multiple visual areas of the primate brain have been found to change their position, before a saccade begins, to the spatial position they will occupy after the saccade (Duhamel et al 1992, Sommer and Wurtz 2006, Neupane et al 2016).While some studies have reported such *forward remapping*, in which receptive fields shift to their postsaccadic locations, others have reported *convergent remapping*, in which receptive fields shift toward the saccade target (Tolias et al 2001, Zirnsak et al 2014). We have found that both mechanisms exist in area V4 (Neupane et al 2016). Here we report new neurophysiological data using an experimental configuration in which both forward and convergent remapping would lead to receptive field shifts in opposite directions. We show that forward remapping is the dominant type of receptive field shift in V4. In a minority of the neurons, receptive fields first shifted towards the forward direction and subsequently reversing direction to follow the convergent direction. Furthermore, remapping seems to require a transient rewiring of position signals matched according to the spatial and temporal properties of each saccade. One candidate mechanism that could support remapping is a change in the coherence of oscillatory LFP signals between distant sites on a retinotopic map. Indeed oscillatory coherence has been shown to support transient rewiring of neural circuits in other contexts (Fries 2015), with different functions being associated with different frequencies. We therefore asked whether the two types of remapping were implemented on a cortical map via coherence at different frequency bands. We recorded from multi-electrode arrays while monkeys performed a saccade task. This allowed us to record simultaneously from two groups of neurons: those encoding the current receptive field and those encoding the post-saccadic future receptive field. We found that the two neural population located on the V4 retinotopic map and separated by a distance specifying the saccade vector, had coherent LFP oscillations in the frequency range of 8-12Hz around the time of saccade execution. At a later time after the saccade, we found coherence in gamma (40-60Hz) frequency range between the neural populations encoding respectively the visual space near fovea and those encoding the periphery. We propose that rapid changes in connectivity on a retinotopic map can be achieved dynamically by changes in synchrony across different neural populations in specific frequency bands.

Disclosures: **S. Neupane:** None. **D. Guitton:** None. **C.C. Pack:** None.

Poster

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Title: Saccade metrics reflect decision-making dynamics during urgent choices

Authors: *J. SEIDEMAN, V. E. SCERRA, E. SALINAS, T. R. STANFORD;
Neurobio. & Anat., Wake Forest Sch. of Med., Winston Salem, NC

Abstract: Saccadic eye movements are perhaps the most ubiquitous manifestation of the perceptual decision and motor choice mechanisms that underlie visually-guided behavior. Once thought to be highly stereotyped and ballistic in nature, there is evidence to suggest that a signature of the decision-making processes that precede a saccade may exist within its metrics and/or kinematics. However, no study to date has provided a precise account of the impact of perceptual information processing on oculomotor dynamics as they unfold over the time course of saccade preparation. We investigated this using a novel urgent-choice paradigm in which the time available to view stimulus information prior to committing to a saccadic choice varied on a trial-by-trial basis. Our findings demonstrate that saccade velocity, amplitude, curvature, and endpoint precision all vary continuously as a function of perceptual processing time, and changes in these metrics track closely the processing-time-dependent changes in overall choice performance characteristic of the task. Interestingly, the amplitude and peak velocity effects observed are not redundant, as perceptual processing time explains variance in the saccadic main sequence. Our findings provide a unique, moment-by-moment, glimpse into how the state of the perceptual decision-making process at the time of choice commitment impacts the execution of that choice. Said differently, these data suggest that the metrics of any given saccade may reflect — to a much finer level of detail than previously thought — the dynamics of the internal cognitive processes that preceded it.

Disclosures: **J. Seideman:** None. **V.E. Scerra:** None. **E. Salinas:** None. **T.R. Stanford:** None.

Poster

717. Eye Movements II

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Topic: E.01. Eye Movements

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DGE 1106400

Title: Beta frequency TMS disrupts top-down cognitive control

Authors: *J. RIDDLE, M. D'ESPOSITO;
Univ. of California Berkeley, Berkeley, CA

Abstract: Neural oscillation theory offers a mechanism of coordinating brain networks for information processing. Neural signals in the beta frequency (20-35 Hz) are proposed to orchestrate top-down cognitive control, while gamma frequency (35-60 Hz) signals are proposed to propagate bottom-up information driven by the environment. Buschman & Miller 2007 tested this hypothesis while monkeys performed a visual search and pop-out saccade paradigm. In this task, a briefly presented cue is remembered over a short delay, followed by an array of 4 targets displayed in the periphery, and the subject is instructed to saccade to the remembered cue item. In the pop-out condition, the distractors in the 4 target array differ from the target in orientation and color. In the visual search condition, the distractors are similar in orientation or color, presumably leading to the need for increased cognitive control. In the pop-out condition, it was found that in gamma frequency signals from parietal cortex to the frontal eye fields (FEF) was associated with bottom-up processing whereas in the visual search condition beta frequency signals from FEF to parietal cortex was associated with top-down processing.
Recent human studies have used rhythmic transcranial magnetic stimulation (rTMS) to disrupt or entrain neural oscillations in a specific frequency band. In this study, we applied 4 pulse trains of rTMS at 50 Hz (gamma) and 20 Hz (beta) to FEF and intraparietal sulcus (IPS) to 20 subjects while they perform the identical paradigm used by Buschman & Miller. On the first day, we collected fMRI data while subjects performed the visual search and pop-out saccade task in order to obtain subject-specific TMS target sites for FEF and IPS. On the following three days, we used rTMS to stimulate right FEF, IPS, and a control region (somatosensory cortex, S1). rTMS was applied at a critical stage for information processing: following the presentation of the target array in the perisaccadic window (just before a saccade is initiated). Beta rTMS was applied 30 ms after the target array appeared whereas gamma rTMS was applied 120 ms after the target array. In both conditions, rTMS was actively applied at the time when criteria for a response is

being formulated as shown in Miller & Buschman 2007.
Following beta rTMS to FEF, as compared to S1, subjects had a faster reaction time and decreased accuracy during the visual search condition only. Our findings provide causal evidence complementing the physiological findings reported by Miller & Buschman 2007, supporting the proposal that beta oscillations emanating from FEF are necessary for cognitive control processes.

Disclosures: J. Riddle: None. **M. D'Esposito:** None.

Poster

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Program#/Poster#: 717.07/OO5

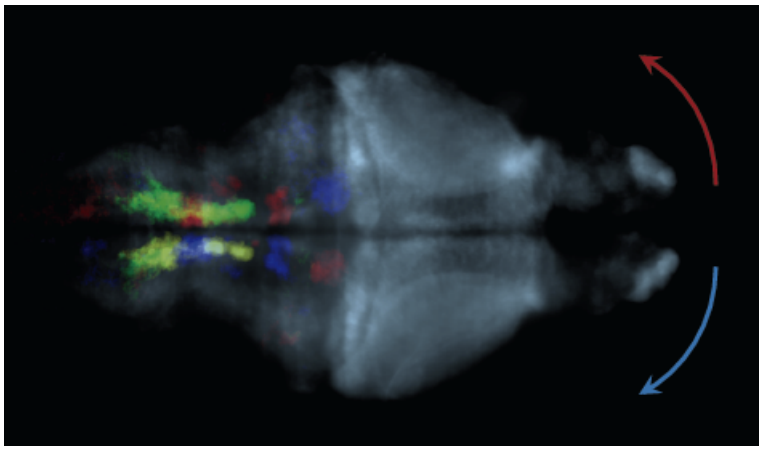
Topic: D.08. Visual Sensory-motor Processing

Title: A visually-entrained saccade command circuit driving phototaxis in zebrafish.

Authors: *G. DEBREGEAS[1], S. WOLF[2], A. DUBREUIL[3], V. BORMUTH[2], R. CANDELIER[2], R. MONASSON[3];
[1]LJP - UPMC/CNRS, Paris, France; [2]Lab. Jean Perrin - UPMC/CNRS, Paris, France; [3]Lab. de Physique Théorique - ENS, UPMC, CNRS, Paris, France

Abstract: In order to survive and thrive, motile organisms use sensory cues to navigate towards favorable environments. Efficient goal-directed locomotion requires a closed-loop coordination between motor action and sensory perception. Each movement induces a new sensory signal, which in turn modulates the forthcoming motor output. This mechanism is at play in a number of taxes behaviors, in organisms ranging from bacteria to humans. Numerous models have been proposed to account for this complex coordinated motion, but to date, no data are available to understand how these behavioral strategies might be implemented at the neural level in the vertebrate brain.
Here we take advantage of the accessibility of zebrafish larvae to whole-brain imaging to identify and dissect the central neural circuit that subserves positive phototaxis. We first established, through behavioral assays, that saccade-induced gaze shifts and reorientation turn bouts are robustly coordinated, and that the statistics of gaze orientation is biased towards illuminated regions. We then used volumetric functional imaging and optogenetic activation to identify the circuit controlling spontaneous gaze dynamics by direct correlation between gaze-shift sequences and neural activity (see figure). We demonstrated that this self-oscillating network is a substrate for visual integration. Its interaction with the visual pathway however differs from a simple stimulus-response pattern. Visual stimuli exert an action that depends on

the circuit oscillatory phase at which they are delivered, a mechanism that manifests itself in the phase-locked entrainment of the circuit by periodic stimuli. We developed a rate-model of this circuit that reproduced most of our observations, and provides the first comprehensive description of how phototaxis can be implemented in the vertebrate brain.
Projection map showing the neural population tuned to ocular saccades. This image was obtained from volumetric light-sheet based functional imaging.



Disclosures: **G. Debregeas:** None. **S. Wolf:** None. **A. Dubreuil:** None. **V. Bormuth:** None. **R. Candelier:** None. **R. Monasson:** None.

Poster

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Title: Visuomotor activity in the frontal eye field is differentially modulated by attentional demands across neuronal types

Authors: *V. SCERRA[1], M. COSTELLO[3], E. SALINAS[2], T. STANFORD[2];
[1]Wake Forest Univ., Winston Salem, NC; [2]Neurobio. and Anat. Dept., Wake Forest Univ., Winston-Salem, NC; [3]Dept. of Neurol., Johns Hopkins Univ., Baltimore, MD

Abstract: Perceptual decision making is the process whereby sensory information is used to guide choices and actions. We studied the dynamic interplay between perception, attention, and motor planning that is required for selecting both the action and the target for that action, and which is particularly crucial when time is limited. These processes are thought to have some correspondence with the characteristic visual (V), visuomotor (VM), and motor (M) cells found in the frontal eye field (FEF). Many tasks used for studying the participation of FEF in perceptually-driven choices require subjects to select an oddball stimulus, allowing for perceptual selection to occur before a motor report is imperative. The compelled saccade (CS) task developed in our lab reverses this order by first issuing a movement imperative and then varying the amount of perceptual information available to guide the decision. By applying the same temporal constraints to both a match-to-sample (CS) and an oddball task (CO), we explored how attentional conditions (top-down vs. bottom-up) impacted behavior and neural activity over a continuum of perceptual processing times. We recorded single-unit activity from characteristic FEF cells (V, VM, M) from non-human primates performing a battery of tasks varying in both urgency and attentional demands. We found that while V cells, traditionally thought to "select" targets independently of motor action, do not discriminate target from distracter in the CS task, they do so in the CO task, indicating that their selectivity is driven by bottom-up attention. We also observed that while motor and visuomotor cells faithfully convey saccadic direction in correct trials, their responses in error trials are substantially correlated with target location, demonstrating a direct impact of perceptual information on their activity. Furthermore, such modulation was stronger in the CO task and weaker — but still present — in the CS task, suggesting that both attentional mechanisms (bottom-up and top-down) are associated with traditional FEF choice-related activity.

Disclosures: V. Scerra: None. **M. Costello:** None. **E. Salinas:** None. **T. Stanford:** None.

Poster

717. Eye Movements II

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Program#/Poster#: 717.09/OO7

Topic: E.01. Eye Movements

Support: Canadian Institutes for Health Research - MOP-130444

Title: Effect of allocentric cues on primate gaze behaviour in a cue conflict task

Authors: *J. CRAWFORD[1], J. LI[1], V. BHARMAURIA[1], A. SAJAD[1], R. MARINO[2], X. YAN[1], S. SUN[1], H. WANG[1];
[1]Ctr. for Vision Res., York Univ., North York, ON, Canada; [2]Ctr. for Neurosci. Studies, Queen's Univ., Kingston, ON, Canada

Abstract: The visual system can remember the location of a peripheral target relative to the self (egocentric coordinates) or to an external landmark (allocentric coordinates). The relative influence of each reference frame have been examined for reach (Byrne & Crawford*, J. Neurophysiol.* 2010), but have not been systematically explored in the gaze control system. Here, we utilized a cue conflict paradigm to assess the effect of allocentric cues on gaze behaviour in the rhesus monkey. Two monkeys (M1 and M2) were trained to maintain central fixation while a target was presented for 100ms in one of eight radial directions, along with an allocentric cue presented at one of four oblique directions 11º from the target. This cue was the intersection of two horizontal/vertical lines spanning the visual field. After a 100ms delay, a mask was shown for 100ms during which the allocentric cue was displaced by 8° in one of eight radial directions. After a second delay of 300-700ms, the fixation point extinguished, acting as a 'go' signal for a head-unrestrained saccade towards the remembered target. To examine the effect of the presence of an allocentric cue on gaze behaviour, the allocentric cue was randomly removed in 50% of control trials where the cue did not shift. Overall, there was a significant ($P < 0.01$) influence of the cue on gaze endpoints, with a mean gain of 0.14 in M1 and 0.17 in M2 (where 0 = target location and 1.0 = cue location). In addition, this cue influence was significantly greater when it is closer to the initial gaze position ($P < 0.01$). In shift conditions, the monkeys did not look toward the original target or the cue location, but rather toward a point shifted partially towards a virtual target defined relative to final cue location (i.e., in allocentric coordinates). Overall, there was a significant ($P < 0.01$) allocentric shift in gaze endpoints relative to controls (with no cue shift), with a mean gain of 0.27 in M1 and 0.23 in M2 (where 0 = no shift and 1.0 = complete shift). In addition, the cue had a significantly greater effect when it is closer to the initial gaze position ($P < 0.01$), when it shifted away from the target ($P < 0.01$), and when it shifted away from the initial gaze position ($P < 0.01$). These findings suggest that internal representations of gaze targets are weighted between egocentric and allocentric cues, and this weighting is further modulated by specific gaze parameters.

Disclosures: J. Crawford: None. **J. Li:** None. **V. Bharmauria:** None. **A. Sajad:** None. **R. Marino:** None. **X. Yan:** None. **S. Sun:** None. **H. Wang:** None.

Poster

717. Eye Movements II

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Program#/Poster#: 717.10/OO8

Topic: E.01. Eye Movements

Title: A model to predict human intention by means of eye movements

Authors: *G. A. ZITO, A. FAISAL;
Dept. of Bioengineering, Imperial Col. London, London, United Kingdom

Abstract: We observe the world through discrete, rapid, focused eye movements acting to align the fovea with an object of interest. Visual information is vital to motor planning and thus monitoring eye movements gives significant insight into our motor intentions. While previous works (Yarbus, Land) focused on demonstrating that eye movement patterns are reflective of specific intentions, we study the inverse problem, to what extent action intention is predictable from eye movements. Thus, we aim to build a computational model that translates highly variable eye-movement patterns into predictions about possible actions. We measure visual exploration behaviour associated with different, well defined, activities of daily living in a table-based scenario. In a first experiment, healthy volunteers are asked to perform a sequence of tasks (e.g. prepare breakfast, eat an apple) while their eye movements are tracked with a head-mounted eye tracker (including scene camera). The various interactions on each object are then associated with a particular pattern of eye movements. Given the high variability of the eye movements, even within restricted task contexts, probabilistic models and pattern recognition algorithms are used for the classification analysis. A Bayesian latent variable model is applied in order to learn the mapping from eye movement features to action. In a second experiment, aiming to validate the developed model, subjects freely perform the same set of tasks in the same scenario. The object they are interacting with is identified based on the analysis of the scene camera. This allows our model to run in a predictive mode, and to label the most likely intention that the subject is going to execute. We report the prediction accuracy of the model against subject actual actions.

Disclosures: **G.A. Zito:** None. **A. Faisal:** None.

Poster

717. Eye Movements II

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Title: Spiking correlations in the frontal eye fields during eye movement planning

Authors: *S. B. KHANNA[1,2], A. C. SNYDER[3], M. A. SMITH[1,2];
[1]Dept. of Ophthalmology, [2]Bioengineering, Univ. of Pittsburgh, Pittsburgh, PA; [3]Carnegie Mellon Univ., Pittsburgh, PA

Abstract: Pairs of nearby cortical neurons exhibit correlated spiking activity. There is a strong link between the correlation among neurons and the amount of information that can be represented in a neuronal population. That link is particularly important at the decoding stage, when sensory signals are used to guide motor output, such as eye movements. Very little is known, however, about the correlated activity in areas that bridge the sensory and motor divide. The frontal eye fields (FEF) are considered to be the primary locus of cortical signals controlling eye movements. Because of this and the presence of neurons with both visual and motor responses, they are an ideal candidate for studying the role of correlated activity in planning and executing movements. Of particular interest is the connection between the populations of visual and motor neurons, which might be important for eye movement planning. We used a linear electrode array to record from groups of FEF neurons in alert rhesus macaque monkeys performing a conventional memory guided saccade task. We measured neuronal correlation of spiking activity on both short and long time scales (spike count correlation and synchrony). We found that correlated spiking activity in FEF was similar in a number of ways, such as dependence on distance and tuning similarity, to previous measurements in early visual cortex. When we focused specifically on connections between visual and motor neurons, we found a distinct pattern of results. The overall level of correlation between these groups was lower than visual-visual or motor-motor pairs, but it showed the strongest dependence on the direction of the planned eye movement. These findings suggest that visual and motor populations of neurons in the FEF play a unique role in transforming visual information to motor output.

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Poster

717. Eye Movements II

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Topic: E.01. Eye Movements

Support: FWF-Grant 20881-B09

Title: Palisade endings in extraocular muscles have an exocytosis machinery

Authors: ***R. BLUMER**[1], J. STREICHER[2], P. J. MAY[3], M. A. DAVIS-LÓPEZ DE CARRIZOSA[4], R. R. DE LA CRUZ[4], A. M. PASTOR[4];
[2]Ctr. of Anat. and Cell Biology,, [1]Med. Univ. Vienna, Vienna, Austria; [3]Dept. of Neurobio. & Anatom. Sci., Univ. of Mississippi Med. Ctr., Jackson, MS; [4]Dept. de Fisiología, Univ. de Sevilla, Sevilla, Spain

Abstract: Proprioception from extraocular muscles (EOMs) provides the brain with eye position signals. Surprisingly, classical proprioceptors (muscle spindles and Golgi tendon organs) are absent in the EOMs of most mammals. Palisade endings have been assumed to be an alternative nervous end organ. These structures are formed by axons which extend into the tendon where they make a u-turn and divide into axonal branches that establish nerve terminals around the tip of a single muscle fiber. Palisade endings are exclusively present in EOMs of mammals including man. All frontal-eyed species exhibit palisade endings whereas they are infrequent and less elaborated in lateral-eyed species. For a century, palisade endings have been considered as sensory structures providing eye position information. The debate on the function of palisade endings has been reopened when recent molecular studies and neuronal tracing experiments have shown that they have a cholinergic phenotype and arise from motor nuclei. Despite these clear motor features, the function of palisade endings is unclear and it is still a matter of debate whether they are sensory or motor or eventually both. In motor neurons, neurotransmitter release requires the presence of the so-called SNARE proteins (SNAP25, synaptobrevin, syntaxin, and synaptotagmin) which mediate fusion of synaptic vesicles with the presynaptic membrane. Here, we tested in monkey and cat if palisade endings contain SNARE proteins as well. Palisade endings were doubly immunolabeled with antibodies against choline acetyltransferase (ChAT)/SNAP25, ChAT/synaptotagmin, ChAT/syntaxin, or ChAT/synaptobrevin. Muscle fibers were labeled with phalloidin. Immunolabeled palisade endings were analyzed in the confocal laser scanning microscope. In accordance with previous studies, palisade endings in cat and monkey expressed ChAT. Moreover, all palisade endings were positive for SNAP25, synaptotagmin, syntaxin, and synaptobrevin as well. Our findings that palisade endings, analog to motor neurons, express proteins of the SNARE complex provide further arguments that palisade endings might function as effector organs.

Disclosures: **R. Blumer:** None. **J. Streicher:** None. **P.J. May:** None. **M.A. Davis-lópez de carrizosa:** None. **R.R. de la CRUZ:** None. **A.M. Pastor:** None.

Poster

717. Eye Movements II

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Topic: E.01. Eye Movements

Support: NIH Grant EY021286

Title: Common motion error correction guides pursuit and fixation

Authors: ***S. N. WATAMANIUK**[1,2], S. J. HEINEN[2];
[1]Psychology, Wright State Univ., Dayton, OH; [2]The Smith-Kettlewell Eye Res. Inst., San Francisco, CA

Abstract: Smooth pursuit and fixation are generally considered to be controlled by different systems. The pursuit system takes motion information at its input and uses a feedback loop to minimize the retinal image motion of a target. Previously, we found that initial pursuit eye acceleration increased as the number of elements in the stimulus increased, implying that more stimulus elements created a stronger motion signal to drive pursuit (Heinen et al., 2016). The fixation system works to keep the eyes on a target, not only with microsaccades, but also with smooth movements. Therefore it, like the pursuit system, may also operate to minimize retinal motion. Might the fixation system, like the pursuit system, also use motion to control the eyes? We hypothesized that if ocular fixation used a motion signal, then increasing the strength of that motion signal should increase ocular stability. To test this, we used fixation stimuli with different numbers of elements to vary the strength of the retinal motion signal created when the eyes moved smoothly across them. Observers fixated either a small, spot target, the center of a 6° circular array of 8 dots, or the center of a 9-dot conglomerate of these stimuli. Eye movements were measured using an EyeLink 1000 eye tracker at a rate of 1000 Hz. We found that the speed of smooth movements decreased as the number of elements in the stimulus increased, paralleling our previous results on pursuit open-loop acceleration. The results provide evidence that the system controlling smooth movements detects the retinal motion of the fixation object, and uses the motion signal that arises to reduce smooth movement speed and stabilize the eyes. We propose that a feedback loop operates during fixation to minimize retinal motion analogous to the feedback loop that minimizes retinal motion during smooth pursuit.

Disclosures: **S.N. Watamaniuk:** None. **S.J. Heinen:** None.

Poster

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Topic: E.01. Eye Movements

Support: NIH Grant R01NS078311

Title: Interaction between effort and reward in the oculomotor system

Authors: *T. YOON, R. SHADMEHR;
Johns Hopkins Med. Inst., Baltimore, MD

Abstract: It is thought that in selecting between available actions, the brain assigns a utility to each possible action and then selects the action that has the highest utility. This utility depends on the subjective value of reward, and the expected effort. The central question is with regard to how the brain measures effort and how this measure interacts with reward.
We have recently shown that the subjective value of a stimulus affects the vigor with which the brain moves the eyes toward that stimulus: stimuli that are valued more coincide with saccades that have higher velocity (Reppert et al. 2015). Here, we investigated the effect of effort by measuring fixation times that separated voluntary saccades.
It is known that during fixation, the total force produced by the opposing extraocular muscles increases with distance from the center position of the eye, as do the number of spikes need to hold the eyes during fixation. During fixation we estimated the sum of spikes produced by the two abducens motor nuclei as an objective measure of energy spent to maintain fixation, and hypothesized that effort is proportional to this sum. This predicted that the effort associated at fixating a stimulus should increase linearly with distance from center. To test for this, we provided human subjects with two images, constant distance apart, but placed at various locations along the horizontal axis. The images were faces (high value) and shapes (low value). We measured time spent fixating one location as a fraction of total time spent at the two locations. Total time spent was constant for every trial throughout the experiment. We found that the ratio of fixation time on one location by fixation time on the other location with constant distance apart decreased linearly as distance of the image increased from center. That is, as the number of spikes required maintaining fixation increased, the time spent looking at that image decreased. The slope of the line was greater when the stimulus value was smaller. These results suggest that the brain assigns utility as an additive function of reward and effort, not multiplicative, and that effort may be associated with the energetic cost of motoneuronal discharge during the action.

Disclosures: **T. Yoon:** None. **R. Shadmehr:** None.

Poster

717. Eye Movements II

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Topic: E.01. Eye Movements

Support: NIH Grant EY014263

Title: Horizontal and vertical premotor gaze populations within the macaque mesencephalic reticular formation

Authors: M. O. BOHLEN[1], S. WARREN[2], *P. J. MAY[2];
[1]Biomed. Engin., Duke Univ., Durham, NC; [2]Neurobio. and Anatom. Sci., Univ. Mississippi Med. Ctr., Jackson, MS

Abstract: It has been proposed that the gaze-related portion of the mesencephalic reticular formation (MRF) can be subdivided into two different regions based on physiological features. Stimulation of and recording from the central mesencephalic reticular formation (cMRF), located caudal to the level of the interstitial nucleus of Cajal (InC), suggests this region is related to horizontal gaze. The peri-interstitial nucleus of Cajal portion of the mesencephalic reticular formation (piMRF), located rostrally, at the level of the interstitial nucleus of Cajal, is believed to play a role in vertical gaze. To test whether this dichotomy is supported by the connectivity of the two regions, we have used dual tracer anatomical experiments in macaque monkeys (*M. fascicularis*). Specifically, to examine the premotor connections of the MRF, we placed anterograde tracers in this nucleus and retrograde tracers in the ipsilateral medial and superior rectus muscles. Experiments in which the anterograde tracer was located within the MRF showed extensive close associations between labeled MRF terminals and labeled medial rectus motoneurons located within the ipsilateral oculomotor nucleus and in C-group, and with labeled superior rectus motoneurons located in the contralateral oculomotor nucleus and in S-group. Ultrastructural examination proved the presence of synaptic contacts between these labeled elements. Looking across the cases used in these experiments, we noted that when anterograde tracer was confined to within the cMRF, there was scant anterograde terminal labeling near superior rectus motoneurons. However, with more rostral injection sites that spread into the piMRF, there was a significant increase in terminals associated with superior rectus motoneurons and a decrease in the terminals associated with medial rectus motoneurons. These differences in preferred motoneuron target suggest that there is a difference in the pattern of connectivity between the cMRF and piMRF that is suggestive of differential function in horizontal and vertical gaze, respectively. Whether this finding reflects an absolute difference between these MRF subnuclei or a continuous spectrum of change between the rostral and caudal poles of the MRF remains to be determined.

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Poster

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Topic: E.01. Eye Movements

Support: BFU2015-64515

BFU2012-33975

FIUS PRJ201402146

Title: The postnatal development of palisade endings in extraocular muscles

Authors: R. BLUMER[1], J. STREICHER[1,2], M. A. DAVIS-LOPEZ DE CARRIZOSA[3], R. R. DE LA CRUZ[3], *A. M. PASTOR[4];
[1]Ctr. of Anat. and Cell Biol., Med. Univ. Vienna, Vienna, Austria; [2]Karl Landsteiner Univ. of Hlth. and Sci., Krems a.d. Donau, Austria; [3]Dept. de Fisiología, Univ. de Sevilla, Sevilla, Spain; [4]Dept. de Fisiología, Univ. De Sevilla, Sevilla, Spain

Abstract: Proprioception from extraocular muscles (EOMs) provides the brain with eye position signals. Surprisingly, classical proprioceptors (muscle spindles and Golgi tendon organs) are absent in the EOMs of most mammals. Instead, palisade endings (PE) are found as unique to EOMs of mammals. They are regularly present in frontal-eyed but infrequent in lateral-eyed species. PE are formed by axons which extend into the tendon where axons make a u-turn and divide into axonal branches that establish nerve terminals around the tip of a single muscle fiber. For a century, PE have been considered as sensory structures substituting classical proprioceptors in EOMs. The interest in PE has newly aroused when molecular analysis and recent neuronal tracing experiments have shown that PE are cholinergic and originate from the EOM motor nuclei. Up to date the development of PE is unknown. Here we have analyzed the postnatal development of PE in cats of different ages (P0, P8, P22, and adult) in all four rectus muscles. In cat P0, eyelids are naturally closed and by P8 eyelids start to open. Immunolabeled PE were analyzed in the CLSM. At P0, no PE were found in both vertical and lateral rectus. Only in the medial rectus we observed axons which extended straight into the tendon where they stopped and extensively sprouted. Other axons extended only a very short distance into the tendon where they made u-turns and formed very simple PE on the tip of single muscle fibers. At P8, eyelids start to open in kittens, PE were still absent in the superior and lateral rectus muscles.

In the inferior rectus muscle, simple PE were found whereas those in the medial rectus muscle were more elaborate but still not mature. At P22, PE were still absent in the superior and lateral rectus. In the inferior rectus and medial rectus, PE were still immature but more complex in the medial rectus. In the medial rectus muscle their number resembled that found in adult cats. In the adult cat, PE were found in each rectus muscle. We have recently shown in frontal-eyed animals that the number of PE is much higher in the medial rectus than in the other rectus muscles suggesting that PE play a role in convergence movements. Since the development of PE is faster in the medial rectus muscle, the present findings supports the notion that PE could play a particular role in convergence. Since the development of palisade endings is more accelerated in the medial rectus muscle, the present findings supports the notion that palisade endings could play a particular role in convergence.

Disclosures: R. Blumer: None. **J. Streicher:** None. **M.A. Davis-Lopez de Carrizosa:** None. **R.R. de la Cruz:** None. **A.M. Pastor:** None.

Poster

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Program#/Poster#: 717.17/PP1

Topic: E.01. Eye Movements

Support: CIHR

Title: Time course for the accumulation of errors in the superior colliculus during memory-guided gaze shift

Authors: *A. SAJAD[1], M. SADEH[2], X. YAN[2], H. WANG[2], J. CRAWFORD[2];
[1]Ctr. for Vision Res., [2]York Univ., Toronto, ON, Canada

Abstract: During a memory-guided gaze shift task, where subjects make delayed gaze shifts towards remembered location of visual stimuli, neurons in the superior colliculus (SC) often show neuronal responses related to visual presentation (visual burst), the evoked gaze shift (motor burst), and the intervening memory delay (delay activity). Previously, we showed that in this task (performed in head-unrestrained conditions), early visual transient response in the SC encodes the accurate location of the visual stimulus (target, T) and the motor burst encodes the final gaze position (G), reflecting the full extent of variability in behavioural errors and this was even observed within single visuomotor (VM) neurons (Sadeh et al., *Eur. J. Neurosci.,* 2015). However, what is unknown is how this T-to-G transition occurs in the SC as activity evolves from vision, to memory delay, to action. In order to investigate this, we analyzed the evolution of

spatial code in 48 visually-responsive neurons from the SC of two monkeys through the entire visual-memory-motor extent of their response. We applied a spatial model-fitting method described previously to identify the spatial code (Keith et al., *J. Neurosci. Methods.,* 2009; Sajad et al., *eNeuro,* 2016): We created a continuum of spatial models (referred to as T-G continuum) that were based on T (target position), G (gaze endpoint), and incremental positions spanning T and G and defined the spatial code as the model that best fits neural data. For each neuron we identified the spatial code during several epochs: early and late visual burst, early and late delay period (obtained by dividing delay period into half), and pre- vs. post-saccadic motor burst. Our individual neuron analysis and population results (30 VM neurons and 18 visual neurons) revealed that the early and late phases of the visual burst both contained a relatively accurate target code, but as activity progressed from the visual burst through memory delay, into the motor burst (in VM cells) there was a progressive transition from T towards G, and this transition was often completed by the time of gaze onset. These results show that the delay activity in the SC does not maintain an accurate representation of the target, but rather maintains a spatial code that is subject to gradual accumulation of errors describing the eventual location of the gaze shifts.

Disclosures: **A. Sajad:** None. **M. Sadeh:** None. **X. Yan:** None. **H. Wang:** None. **J. Crawford:** None.

Poster

717. Eye Movements II

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Program#/Poster#: 717.18/PP2

Topic: E.01. Eye Movements

Title: Translating choice to smooth pursuit eye movements in the frontal eye field

Authors: *M. JOSHUA;
The Edmond and Lily Safra Ctr. for Brain Sci., The Hebrew Univ. of Jerusalem, Jerusalem, Israel

Abstract: Action selection and its planning are critical for controlling the environment. We designed this study to characterize the neural dynamics during planning of target selection and link the activity to selection-dependent and selection-independent behavior. We recorded activity in the smooth pursuit area of the frontal eye field (FEF) while monkeys were planning a smooth pursuit eye movement that selected one of two moving targets. The most common preparatory neural activity in the FEF was ramping activity preceding target motion onset, which was linked on a trial-by-trial basis to pursuit eye movement latency rather than to its direction. Pursuit

selection was encoded by the offset of this ramping response, relative to baseline. Planning of selection was commensurate with a winner-take-all representation rather than a representation that matched the specifics of the upcoming movement. These findings characterize the majority of the dynamics in the average activity and show that in planning selection the FEF mainly signals when to move and not where to move.

Disclosures: M. Joshua: None.

Poster

717. Eye Movements II

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Topic: D.08. Visual Sensory-motor Processing

Support: NIH Grant EY03878

Title: Representation of Bayesian priors for target speed in the preparatory activity of neurons in the smooth eye movement region of the frontal eye fields

Authors: *T. DARLINGTON, S. LISBERGER;
Dept. of Neurobio., Duke Univ., Durham, NC

Abstract: Many behaviors operate in a Bayesian framework, where actions are guided by a complex interaction between current sensory information and past experience, or “priors”. When current sensory information is weak, it is advantageous to allow the prior to guide behavior. However, the value of prior experience lessens as sensory information strengthens. Despite the generalizability of this phenomenon, it remains unclear how Bayesian estimation is implemented at the level of neural circuits. Smooth pursuit eye movement is an example of a relatively simple sensory-motor behavior that operates in a Bayesian-like manner. We have developed a paradigm that allows for the fast adaptation and probing of Bayesian priors for target speed. Two male rhesus macaque monkeys were trained to track a 100% contrast patch of dots and a 6% contrast sine wave grating with smooth pursuit eye movements. We used a block design to adapt priors for target speed. During the “fast prior” block, targets moved at 20 deg/s for 80% and at 10 deg/s for 20% of the trials. During the “slow prior” block, targets moved at 2 deg/s for 80% and at 10 deg/s for 20% of the trials. During a control block, the targets moved at 10 deg/s for 100% of the trials. Compared to the control block, we find that eye speeds for the 10 deg/s target motion are faster in the fast prior block and slower in the slow prior block. Furthermore, the change in eye speed is greater for the low contrast target (weak motion) compared to the high contrast target (strong motion), consistent with the Bayesian framework. We have used our paradigm for rapid

prior adaptation to ask whether preparatory activity in the smooth eye movement region of the frontal eye field (FEFsem) encodes information about the prior for target speed. Our population of cells in both monkeys exhibited preparatory ramps in firing rate during fixation, prior to visual motion onset. We observed cells with both increasing and decreasing ramps in activity. The magnitude of the ramps was larger during the fast prior block compared to the slow prior block. We conclude that preparatory activity in FEFsem encodes expectation (a.k.a. the prior) for upcoming target speed.

Disclosures: T. Darlington: None. **S. Lisberger:** None.

Poster

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Topic: E.01. Eye Movements

Support: NIH R01-EY014885

Title: Saccade curvature in sequences of voluntary saccades suggests they are not planned independently

Authors: *R. AZADI, R. M. MCPEEK;
SUNY Col. of Optometry, New York, NY

Abstract: Saccades often have curved trajectories, and previous studies have shown that covert attention and the onset of visual distractors can alter this curvature. Recently we showed that saccadic curvature is modified in systematic ways during sequences of saccades. Specifically, each saccade tends to curve in the direction of the preceding saccade vector and opposite to the direction of the following saccade vector in the sequence. However, these findings are based on studies of reflexive and instructed saccades, rather than free-viewing voluntary (exploratory) saccades. Here we used saccadic curvature as a tool to study planning of sequential saccades in a free-viewing visual search task.
In each trial, eight targets (0.8° diameter rings) appeared in random positions on the screen. A horizontal line (0.15° long) was located at the center of one of the rings, while the other seven contained vertical lines. Subjects were instructed to locate the target containing the horizontal line as quickly as possible. To perform the task, the subjects needed to foveate the targets one by one, while eye position was recorded binocularly.Offline analyses showed that saccadic curvature has a sinusoidal relationship to the angle of the saccadic vector, relatively independent of the saccade amplitude, as shown previously in studies of reflexive saccades (e.g., Smit and

van Gisbergen 1990). This relationship between curvature and saccade angle was binocular and similar in both eyes, implying that the curvature is neural in origin, rather than due to anatomical and muscular properties of the orbit. More importantly, the results showed that saccade curvature is modified by the direction of preceding and following saccades: saccades tended to curve in the direction of the immediately preceding saccade vector, and opposite to the direction of the immediately following saccade vector, consistent with our previous findings. Furthermore, the magnitude of this curvature gradually varied based on the angle of the following or preceding saccades.These findings suggest that voluntary exploratory saccades in this task are not processed independently, since the trajectory of each saccade is influenced by the direction of the preceding and following movements in the sequence.

Disclosures: **R. Azadi:** None. **R.M. McPeek:** None.

Poster

717. Eye Movements II

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 717.21/PP5

Topic: D.08. Visual Sensory-motor Processing

Support: NIH Grant EY024831

Title: Insights into sensorimotor transformation in the superior colliculus through current-source density analysis

Authors: *C. MASSOT, U. K. JAGADISAN, N. J. GANDHI;
Eye and Ear Inst., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: The superior colliculus (SC) is crucial for transforming sensory signals that register a target into motor commands that produce an orienting movement to the stimulus. The sensory response is represented as a burst of activity in visual and visuomotor neurons in the superficial and intermediate/deep (collectively, deeper) layers. Saccadic eye movements are produced by yet another burst of activity in the visuomotor and motor neurons in the deeper layers. However, the underlying input signals that produce this pattern of activity are not well understood. We address this gap in knowledge by recording spikes and local field potentials (LFPs) from a 16-channel laminar probe in the SC of a monkey performing randomly interleaved delayed, visually-guided and memory-guided saccades. The electrode penetration was orthogonal to the SC, hence the optimal target locations and/or saccade vectors were comparable across all recording contacts. The target was positioned either close to the center of the response field or at the diametrically opposite location. Here, we quantify LFP information with current-source density (CSD)

analysis to emphasize the location and timing of incoming (source) and outgoing (sink) electrical currents across layers. Preliminary analyses reveal the following observations: 1. The sensory burst is coincident with a robust current source signal in the intermediate layers , with bleeding into the superficial layers. The magnitude of this source decreases gradually during the delay period and then increases modestly at the time of saccade onset. 2. In contrast, a current sink was observed deeper in the SC, at sites of visuomotor spiking activity. This CSD switched to a weak source signal during the delay period, before re-transitioning to a sink at the time of saccade onset to reveal a transient source/sink reversal between intermediate and deep layers. 3. Intriguingly, the CSD trace in the deep layers revealed a potent source signal immediately following the saccade. This cannot be a visual signature since it was also observed for memory-guided saccades. Across all layers, modulations in both LFP and CSD signals during the delay and presaccadic periods were weak compared to the fluctuations observed during sensory and post-saccadic epochs. Taken together these results show key differences between the target and the motoric burst and reveal that each SC layer is involved in different local and global network activity during sensorimotor transformation.

Disclosures: **C. Massot:** None. **U.K. Jagadisan:** None. **N.J. Gandhi:** None.

Poster

717. Eye Movements II

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 717.22/PP6

Topic: E.01. Eye Movements

Support: NIH Grant EY021286

Title: Are catch-up saccades and microsaccades siblings?

Authors: ***S. J. HEINEN**[1], S. N. J. WATAMANIUK[1,2];
[1]Smith-Kettlewell Eye Res. Inst., San Francisco, CA; [2]Wright State Univ., Dayton, OH

Abstract: Catch-up saccades are small saccades that frequently interrupt the smooth component of ocular pursuit. Microsaccades are small saccades that occur frequently during ocular fixation. There is evidence that pursuit and fixation are different neural systems, but are the small saccades that are virtually omnipresent during these ocular behaviors generated by different mechanisms? Here we test this by capitalizing on a curious phenomenon that occurs with microsaccades, notably that before high-acuity tasks, their rate predictably subsides until they are almost completely quieted, and then rebounds. Here we ask if a similar phenomenon occurs for catch-up saccades during pursuit. Observes pursued a linear array of 15 small alphanumeric

characters (0.28° × 0.5°), and performed a character discrimination task on them (Lovejoy et al., 2009). Stimuli appeared stationary for a random fixation duration, then translated either leftward or rightward across the screen at 8°/s, 12°/s, or 16°/s for 1640-2440 ms. At a random time after the fixation period (1040-1440 ms), the character array changed from 8's to 2's and 5's except for a single probe character that changed to a 3 or an E. After 200 ms, all characters changed back to 8's. Observers identified the 3 or E with a keypress. We found that catch-up saccade frequency decreased in prediction of the upcoming discrimination task, and rebounded after the task was completed in a similar fashion to what occurred during fixation. The results provide evidence that the mechanism generating catch-up saccades during pursuit also generates microsaccades during fixation.

Disclosures: S.J. Heinen: None. **S.N.J. Watamaniuk:** None.

Poster

717. Eye Movements II

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Program#/Poster#: 717.23/PP7

Topic: D.08. Visual Sensory-motor Processing

Support: NIH Grant EY024831

Title: Analysis of spiking activity and local field potentials reveals patterned information flow within the superior colliculus

Authors: L. J. DRNACH, *U. K. JAGADISAN, C. MASSOT, N. J. GANDHI;
Dept. of Bioengineering, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: The superior colliculus (SC) is a major hub of sensorimotor integration in the gaze control network, and plays a pivotal role in the generation of saccadic eye movements. The sensory-to-motor transformation is enabled by the intermediate and deep (collectively, deeper) layers of the SC. However, it is unknown whether neurons in these layers constitute a homogeneous network performing similar computations or if there exists finer spatiotemporal patterning therein. To study this in greater detail, we combined linear microelectrode array recordings with multi-channel signal analyses. Linear arrays are especially amenable to recording from a column of neurons to access computations evolving in parallel within the column. We recorded from the SC in two monkeys (Macaca mulatta) performing a delayed saccade task. The electrode contacts (n=16) spanned the dorso-ventral extent of the SC, allowing for the simultaneous recording of spiking activity and local field potentials (LFPs) within the deeper layers.

We performed coherence and Granger causality analyses to assess the flow of information within SC. We found the following:
(1) Following target onset, spike-spike coherence increased between most channel pairs, but only the dorsally located channels exerted a Granger causal influence on the spiking of other channels, suggesting a unidirectional flow of information during sensory processing. In contrast, during the saccade, the middle channels exerted a causal influence on channels located both dorsally and ventrally, indicating bidirectional information flow during peri-saccadic processing.
(2) Spike-LFP coherence revealed stronger coherence between spiking activity recorded from dorsal contacts and LFPs recorded more ventrally, both following stimulus onset and following the saccade. Intriguingly, there was no significant increase in coherence in the lead up to the saccade.
(3) For both epochs, the spike-LFP coherence profile was biphasic, with an early, narrow transient, and a late, broader peak. Granger analyses suggested that both early coherence peaks could be the result of causal dorsal-to-ventral influence of spikes on LFP, whereas the late peaks could be the result of the causal influence of LFP on spikes.
(4) In all cases, coherence and causality decreased as a function of distance between the pair of contacts. Moreover, the strongest influences in all cases were in the sub-beta band (<30 Hz), with a slightly weaker effect in the low-gamma range (30-50 Hz) between dorsal channels.
These results point to distinct communication channels for spikes and LFPs in the SC, and provide evidence for multi-phase processing during sensorimotor integration.

Disclosures: L.J. Drnach: None. **U.K. Jagadisan:** None. **C. Massot:** None. **N.J. Gandhi:** None.

Poster

717. Eye Movements II

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 717.24/PP8

Topic: E.01. Eye Movements

Title: Role of cerebellar nuclei in visual preference for face image and its relevance to autism

Authors: *D. DZIOBEK[1], S. ZHANG[2], J. ASHE[3,5], X. LU[4];
[1]Grad. Program in Neurosci., Univ. of Minnesota Twin Cities, Minneapolis, MN; [2]Dept. of Biomed. Engin., [3]Dept. of Neurosci., [4]Dept. of Neurol., Univ. of Minnesota, Minneapolis, MN; [5]Neurol. Service, Veterans Affairs Med. Ctr., Minneapolis, MN

Abstract: The cerebellum has a clear role in the generation of saccadic eye movements. The 'oculomotor vermis', through its projections to the fastigial nucleus, modulates the amplitude of

saccades. The discovery of projections from the medial cerebellar nuclei to cognitive areas in frontal cortex suggests that these nuclei contribute to cognitive functions in unknown ways. Our particular interest was in testing the hypothesis that neural activity in the cerebellum biases the choice of visual targets of saccades toward socially-relevant cues. Healthy human and non-human primate subjects show a strong preference for socially-relevant images, and we recently demonstrated that monkeys, when given a choice in viewing visual images, show an overwhelming preference for facial images of primates. Such behavior is in stark contrast to that of subjects with autism, in whom damage to Purkinje cells is the most common neuropathological finding, who show no bias toward socially-relevant images and in fact may prefer viewing images of inanimate objects. Functional imaging of autistic individuals during eye movement tasks show reduced activation of the Supplementary Eye Fields (SEF), a region associated with cognitive control of eye movements.
Here we trained a monkey on a face image-based oculomotor task in which he had to choose between objects and images of human and non-human faces and unilaterally inactivated different portions of the cerebellar nuclei by injecting a small amount of muscimol. In control experiments before the inactivation studies, the subject had demonstrated a 95% preference for images of faces compared with images of other objects. We found that the preference rate for face image decreased significantly (Z-test, p=0) following inactivation of portions of the ventral cerebellar dentate nucleus (VDN) and the posterior interpositus nucleus (PIN), to 84% after the injection into the VDN, and 75% after injection into the PIN. There was no change in the preference after the injections into dorsal dentate nucleus and anterior interpositus nucleus, and there was no obvious difference in the preference of pairs of non-face images after injection into each portion of the cerebellar nuclei. These results suggest that the cerebellar nuclei, especially the VDN and PIN, contribute to production of saccades to images of primate faces when given a choice of two images to look at. Together with our finding of cerebello-thalamo-SEF pathway, we hypothesize that cerebellum and its projections to the SEF are involved in generation of eye movements towards primate faces, and failure of this circuit may result in the lack of eye movement towards faces associated with autistic behavior.

Disclosures: **D. Dziobek:** None. **S. Zhang:** None. **J. Ashe:** None. **X. Lu:** None.

Poster

717. Eye Movements II

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Program#/Poster#: 717.25/PP9

Topic: E.01. Eye Movements

Support: NIH NEI R01 EY017707

Title: Pre-existing visual and oculomotor factors that determine the retinal location of a preferred retinal locus (PRL)

Authors: *H. MAZYAR[1], B. S. TJAN[1,2];
[1]Neurosci. Grad. Program, [2]Dept. of Psychology, USC, Los Angeles, CA

Abstract: Diseases such as age-related macular degeneration (AMD) selectively affect foveal and parafoveal vision, leading to central field loss (CFL) and severely impairs functional vision. More than 10 million people in the U.S. suffer from AMD. This number is expected to increase to 21.6 million by 2050[1]. To adjust to CFL, the visual system often adopts an extra-foveal retinal location, called the preferred retinal locus (PRL), to use for fixations. In many patients, saccades are re-referenced to the PRL. The retinal location of a PRL varies across patients. It is not known what visual or oculomotor factors may underlie the development of PRL since measuring these factors before the formation of a PRL had not been feasible. We developed a method that constrains PRL formation to a one-dimensional contour in normally sighted participants. This allows us to comprehensive survey the visual and oculomotor factors prior to PRL formation.

We use the "Contact task" to induce a PRL on a designated one-dimensional contour in normally sighted participants. Participants had to use their gaze to move an opaque and gaze-contingent disc to make contact with a small target. The disc was 6° in radius and centered at the fovea. In each trial, a small target appeared at a random screen location. The participant had to establish contact between the edge of the disc and the target and maintain contact for 0.5 s before the target was dismissed and a new trial began. Before and after performing the Contact task, participants were assessed for their form vision (visual acuity, crowded visual acuity, positional uncertainty) and oculomotor performance (peripherally guided fixation stability and saccade accuracy, number of saccades required to establish peripheral fixation) at 8 evenly spaced locations at 6° eccentricity.

We found that PRL tended to form at a retinal location where it took the participant the least number of saccades to establish peripheral fixation. The retinal location with the least amount of positional uncertainty also facilitated PRL formation. Our results also showed that PRL formation correlated with improvements in visual and oculomotor performance. After PRL formation, the first saccade landing error for establishing peripheral fixation and the number of saccades required were reduced. A slight improvement of crowded acuity was also observed. These findings explain the idiosyncrasy of PRL formation across participants and inform the designs of customized rehabilitation regimens for patients with CFL.

[1]. Rein, D.B. (2009). Forecasting Age-Related Macular Degeneration Through the Year 2050: The Potential Impact of New Treatments. Arch. Ophthalmol. *127*, 533.

Disclosures: H. Mazyar: None. **B.S. Tjan:** None.

Poster

717. Eye Movements II

Location: Halls B-H

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Topic: D.06. Vision

Support: Research to Prevent Blindness

EyeSight Foundation of Alabama

Title: Increased crowding in parafoveal vision in glaucoma

Authors: ***M. KWON**[1], R. LIU[1], C. GIRKIN[2];
[2]Dept. of Ophthalmology, [1]Univ. of Alabama at Birmingham, Birmingham, AL

Abstract: Crowding, the deleterious influence of nearby items on visual recognition, impairs a person's ability to recognize a target object in clutter (Bouma, 1970; Levi, 2008). A popular explanation of crowding is that features of the target and flankers are integrated inappropriately, as they both fall in the same neural unit due to a large integration field (Pelli et al., 2004). Thus, crowding grows with increasing retinal eccentricity as receptive field size increases (i.e. scale shift) in peripheral vision, while little crowding exists in parafoveal or foveal vision. Glaucoma, a leading cause of irreversible vision loss in the United States, is associated with the loss of retinal ganglion cells. Studies have shown that threshold spatial resolution becomes considerably larger as retinal ganglion cell density decreases (Wall et al., 1991). While this evidence hints that the loss of retinal ganglion cells might bring about a larger integration field, which in turn exacerbates the crowding effect, little is known about visual crowding in glaucoma. Here we report crowding in glaucomatous vision in comparison with age-matched normal vision. Crowding was assessed in 15 individuals with moderate glaucoma (mean Mean Deviation for the worse eye: = −11.58 dB±9.1) and 10 age-matched normal controls. Crowding was quantified as the center-to-center distance (crowding zone) between the target and flankers that yielded a target-identification accuracy of 80%. Measurements were made at 9 different retinal locations (retinal eccentricities: 0°, 2° and 4°). In each trial, a subject was presented with five letters, a target flanked by four tumbling Es, and asked to report the target identity. Our results showed that people with glaucoma had a significantly larger crowding zone, even in parafoveal vision (a decrease by 25%, $p < 0.05$), compared to normal cohorts. However, no significant difference was found in single letter recognition between the two groups ($p > 0.05$), suggesting that reduced acuity cannot account for the increased crowding. Our findings suggest that, contrary to the conventional view that central vision remains intact until the very last stage of glaucoma, the macular region of even moderate glaucoma appears to suffer from unusually large crowding.

Disclosures: **M. Kwon:** None. **R. Liu:** None. **C. Girkin:** None.

Poster

717. Eye Movements II

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Topic: D.08. Visual Sensory-motor Processing

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ARC Discovery Projects DP140100266

Title: From eyes to hands – Inhibition training transfers across motor systems

Authors: *A. VERGHESE;
The Univ. of Queensland, St. Lucia, Australia

Abstract: Inhibition of irrelevant information and motor responses is crucial for goal-directed behaviour. Given the importance of inhibitory processing for adaptive functioning in our daily lives, and its impairment in many psychiatric and neurological conditions, a key question is the extent to which this ability can be enhanced via behavioural training. While studies on training of inhibitory processes have consistently shown improvements on the trained task, it is not clear whether such benefits generalize to distinct (untrained) inhibition tasks. Training-dependent modulations of activity in cortical regions such as the right inferior frontal gyrus, precuneus, inferior parietal lobule and the anterior cingulate cortex have been consistently reported in studies of inhibitory control of eye-movements and manual inhibition measures. Since generalized training benefits for executive functions should be observed when the training and transfer tasks tap a common underlying mechanism, it is conceivable that training the same inhibitory process but using different effector systems – eyes versus hands – could evoke training-induced benefits that generalise. Here we assessed whether training of inhibitory control on an anti-saccade task, in which pre-potent oculomotor responses must be inhibited, transferred to other inhibition tasks in which pre-potent manual responses had to be altered. Specifically, we tested whether the well-known 'Simon effect', characterized by longer manual responses to stimuli in non-corresponding spatial locations, and the 'Stroop effect', characterised by longer responses for stimuli with featural conflict, can be modulated by inhibitory oculomotor training. Importantly, we included two active-control training regimens - fixation training and pro-saccade

training. All three training regimens led to improvements on the trained tasks themselves, as expected, but only anti-saccade training yielded benefits that transferred to the manual response modality. Specifically, anti-saccade training led to reductions in the Simon effect, but did not influence the Stroop effect. Taken together, these findings suggest that training of inhibitory control within the oculomotor system can transfer to the manual motor system, and provide important insights into the boundary conditions necessary for transfer of inhibition training.

Disclosures: A. Verghese: None.

Poster

717. Eye Movements II

Location: Halls B-H

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Program#/Poster#: 717.28/PP12

Topic: D.08. Visual Sensory-motor Processing

Support: HHMI

NIH EY011378

NARSAD YI Fellowship

Title: A diffusion process underlies action selection in an abstract decision-making task.

Authors: *S. SHUSHRUTH[1,2], M. SHADLEN[1,2,3];
[1]Neurosci., Columbia Univ., New York, NY; [2]HHMI, New York, NY; [3]Zuckerman Mind Brain Behavior Inst., New York, NY

Abstract: When monkeys and humans form perceptual decisions that lead to particular actions, the accumulation of sensory evidence and the planning of motor actions are yoked. If the evidence is acquired before the decision maker is informed of the action required to report a choice, then these two processes are dissociated. In such situations, an abstract representation of the decision is purportedly stored and this representation subsequently used to drive action selection. How such abstract representations can flexibly recruit relevant motor actions has not been explored.
We trained a monkey to decide on the net direction (left or right) of dynamic random dot motion (RDM) and to associate these directions with a color (yellow and blue, respectively). Importantly, the colored choice-targets appeared at random locations in the visual field 0.33s after the offset of the RDM. Although the monkey could report its decision with an eye movement to the blue or yellow target as soon as they appeared, the observed saccadic latencies were prolonged and showed strong inverse correlation with the strength of the evidence

presented earlier in the trial. The dependence of saccadic latency on the motion strength was fit with a bounded drift-diffusion model, and this fit predicted the monkey's choice behavior. Thus, the prolonged latencies are consistent with a deliberative process involving evidence accumulation after the RDM had been shown and extinguished. Consistent with this idea, the rate of rise of the neural activity in area LIP on individual trials during the action selection epoch was correlated with the strength of the perceptual evidence presented earlier in that trial. Further, the evolution of variance and the autocorrelation of the neural activity during this epoch exhibited signatures of a diffusion process. The responses of the same neurons during the RDM viewing epoch displayed none of these features.
Both the behavioral and the neural data suggest that action selection is not a one step conversion of an abstract representation of the decision into an action but is instead a process that evolves over time in a manner that reflects the quality of the evidence received. An intriguing possibility is that the brain stores a representation of the samples of the evidence acquired during the RDM viewing epoch and then accumulates evidence from these samples to reach a decision after the targets are revealed. If so, the decision appears to be postponed until it can be embodied in the framework of the available motor actions.

Disclosures: **S. Shushruth:** None. **M. Shadlen:** None.

Poster

717. Eye Movements II

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 717.29/PP13

Topic: D.08. Visual Sensory-motor Processing

Title: Temporal change of position representation of a moving object filled with Gabor motion for saccadic eye movements

Authors: *H. UEDA;
NTT, Atsugi-Shi, Japan

Abstract: To interact with a moving object in the environment, it is necessary to know the precise location of the object. However, if a moving object containing internal motion (e.g., rotation) is seen in the visual periphery, the perceived global motion of the moving object is often distorted due to its local motion signals (*motion-induced position shift*). On the other hand, it has been reported (Lisi & Cavanagh, 2015) that eye movements can still correctly localize such a moving object without being affected by the distorted perceived global motion. The study raises two questions: 1) whether the distortion of global motion perception is attributed solely to motion perception or also to position perception, and 2) when target position representation for

eye movements changes from the distorted perceived location to the actual or physical one. In the present study, a well-known curveball illusion, which deviates the actual trajectory of a moving Gabor patch substantially towards the direction of its internal pattern motion, was utilized to examine the temporal change of the position representation of such moving target containing a motion for saccadic eye movements. To this end, we first examined whether this misrepresentation of the global motion trajectory of a moving Gabor patch is attributed to the distortion of its perceived position as well as perceived motion. In the experiment, a moving Gabor target with internal envelope drifts in the orthogonal direction to the global motion appeared at 10 degrees of visual angle to the right of the fixation point and disappeared at random locations on the moving path. Participants localized the final horizontal target position by judging the position was on either the left or right side of a reference line presented shortly afterward (i.e., 2-AFC task). The results confirmed that the moving stimuli containing internal motion induce not only large shifts of perceived motion but also perceived position. In the second experiment, the temporal change of position representation (i.e., motion-induced position shift confirmed in Experiment 1) for saccadic eye movements was examined by varying the elapsed time from target offset to saccade onset. With the same target stimuli as Experiment 1, participants made saccades to the final target position on the motion path after a variety of time-delay. The results showed that, in addition to the previous observation, even when target disappeared 1s before saccade initiation (i.e., memory guided saccade), eye movements could still correctly localize physical target positions. This suggests that a common position representation of a moving object is used for *visually guided* and *memory guided* saccades.

Disclosures: H. Ueda: None.

Poster

717. Eye Movements II

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 717.30/PP14

Topic: D.08. Visual Sensory-motor Processing

Title: Pre-saccadic mislocalization against saccade direction

Authors: *M. LAPPE[1], T. WATSON[2];
[1]Inst. for Psychology, Muenster, Germany; [2]Sch. of Social Sci. and Psychology, Western Sydney Univ., Penrith, Australia

Abstract: Stimuli flashed within 50 ms before the onset of a saccade appear shifted into the saccade direction and compressed onto the saccade target. The compression could result from an oculomotor feedback of the saccade command that distorts the population activity in visual areas

and appears to shift visual receptive fields. In the present study, we report that stimuli flashed about 100 ms before a saccade are mislocalized in the opposite direction, against the saccade and towards the fixation point. Subjects reported the apparent location of flashes presented on a computer monitor in a dimly lit room while making saccades. Flashes could occur at 5 positions equally spaced between the fixation point and the saccade target and were presented at a random time between 300 ms before saccade onset and well after the saccade. Different sessions tested different paradigms: overlap saccades, step saccades, double step return saccades, and fixation with masking at various ISIs. All saccade conditions showed mislocalization against the saccade direction at around 100 ms before the saccade. This was followed by the familiar pattern of compression towards the target at saccade onset. No mislocalization was observed in the fixation with masking condition. Mislocalization against the saccade direction was strongest for flash positions in the middle between fixation point and target. These findings have important implications for models of peri-saccadic localization and remapping. Since the mislocalization occurs outside the time window of saccadic suppression it is not related to a reduction of visibility. It is also unlikely to be related to an erroneous eye position signal. We propose that the mislocalization towards the fixation point results from enhanced fixation related neural activity during preparation of the saccade motor plan.

Disclosures: **M. Lappe:** None. **T. Watson:** None.

Poster

718. Cerebellar Networks and Functions

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

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Topic: E.02. Cerebellum

Support: NIH Grant EY012814

Title: Distinct neuronal dynamics of head tilt and translation Purkinje cell signals in the vestibular cerebellar cortex.

Authors: *J. LAURENS, H. MENG, D. E. ANGELAKI;
Neurosci., Baylor Col. of Med., Houston, TX

Abstract: Brainstem-cerebellar circuits resolve a sensory ambiguity originating from Einstein's equivalence principle, where linear accelerations (A) during translations are physically identical to gravitational accelerations (G).
The underlying computations have been well characterized at a theoretical level. The brain uses an internal model of motion which integrates rotation velocity (Ω) signals into an estimate of tilt

relative to gravity (G), which is used to extract an acceleration estimate (A) from the net otolith signal (F) based on the physical equation F = G+A. This process can be broken down in three equations: (1) dG/dt = GxΩ, (2) G=∫dG and (3) A = F-G. Equation (1) converts a head-referenced rotation velocity signal (Ω) into a gravity-referenced signal dG, i.e. it is a spatial transformation. Equation (2) is an integration that matches the dynamics of the gravity signal with that of F.
We showed previously that the simple spike (SS) responses of one third of Nodulus-Uvula (NU) Purkinje cells (PC) encode G ('tilt' cells), another third encode A ('translation' cells), while the rest ('other') respond to combinations thereof. However, we never investigated the dynamics of tilt and translation signals. Therefore, we couldn't determine if steps (1) and (2) are performed in a single step of neuronal computation or in two distinct neural populations. Furthermore, a subpopulation of otolith afferent carry significant jerk (dF) components, suggesting that equation (3) could be replaced by dA=dF-dG, eliminating the need for equation (2).
Our previous studies were based on sinusoidal stimuli that provide little information about neuronal dynamics. To circumvent this limitation, we recorded the responses of 46 NU PC during combinations of transient tilt and translation stimuli where linear acceleration (A) and tilt (G) followed biphasic profiles while dA/dt and dG/dt followed triphasic profiles. We found that 13 cells responded specifically to tilt and 18 to translation. Strikingly, tilt-selective cells exhibited a triphasic response profile proportional to dG, whereas the response of translation-selective cells was biphasic, proportional to A.
These results suggest that a dG/dt signal (equation 1) is computed by a neuronal population upstream of the Purkinje cells, and that a distinct group of interneurons perform the temporal integration (equation 2). This illustrates how a systematic study of neuronal dynamics through the cerebellar circuitry may allow deciphering the neuronal implementation of a theoretically well understood computation.

Disclosures: **J. Laurens:** None. **H. Meng:** None. **D.E. Angelaki:** None.

Poster

718. Cerebellar Networks and Functions

Location: Halls B-H

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Program#/Poster#: 718.02/PP16

Topic: E.02. Cerebellum

Support: the Medical Research Center, 2012R1A5A2A44671346 from the National Research Foundation of Korea.

Title: Bi-directional modulation of purkinje cell spike activity upon arousal *In vivo*

Authors: *S. HUR, S. J. KIM;
Dept. of Physiol., Seoul Natl. University, Col. of Med., Seoul, Korea, Republic of

Abstract: The cerebellum receives noradrenergic (NA) fibers originating from the locus coeruleus (LC). In the state of arousal, an overflow of this neurotransmitter are diffused and acts on α- and β-adrenergic receptors of neurons modulating the spike activity and synaptic strength. The cerebellum has a well-recognized role in maintaining motor coordination. Many studies regarding cerebellar learning has suggested that by recognizing neural patterns the cerebellum predicts optimal movements thus tuning fine motor movements. The Purkinje cell (PC), the sole output of the cerebellum, receive excitatory and inhibitory inputs from parallel fibers, climbing fibers, and molecular layer interneurons which is summated and relayed to the post-synaptic neuron by simple (SS) and complex spikes (CS) ultimately inducing motor movements. *In vitro* and *in vivo* studies of animals under anesthesia have shown bath and local application of NA excite or depress PC spontaneous spike activity depending on NA concentration. Several reports have introduced change in awake *in vivo* calcium dynamics upon arousal in various cells and regions of the brain. However, how NA effects SS and CS activity of PCs in awake mice upon natural endogenous secretion by arousal has not yet been discussed. Using *in vivo* single-unit electrophysiological recordings in awake C57BL/6J mice, SS and CS activity were recorded from PCs. Recordings began when the mice were in a stable state (low respiration, no locomotion, and stable pupil diameter). To induce arousal, air puff stimulation to the tail evoke a startle response (high respiration, locomotion, and increased pupil diameter). Results show that increase of NA diffused from the LC to the cerebellum show a bi-directional modulation of SS activity upon a startle response but no change in CS activity.

Disclosures: S. Hur: None. **S.J. Kim:** None.

Poster

718. Cerebellar Networks and Functions

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 718.03/QQ1

Topic: E.02. Cerebellum

Support: UT Undergraduate Research Fellowship

MH46904

Title: Variable single unit responses in the pontine nuclei during auditory evoked eyelid conditioning

Authors: *E. A. KISH, M. DODLA, E. D. DELORD, H. E. HALVERSON, M. D. MAUK; Ctr. for Learning and Memory, Univ. of Texas at Austin, Austin, TX

Abstract: The cerebellum makes predictions about what the correct movement is in response to the current state of the world. These predictions can be modified by the repeated coincident arrivals of a sensory stimulus and a teaching signal. This can be described as a Pavlovian learning paradigm where the sensory stimulus is the conditioned stimulus (CS) and the teaching signal is the unconditioned stimulus (US). The CS is conveyed to the cerebellum through mossy fibers that originate from the pontine nuclei. In an effort to elucidate what the cerebellum sees in order to learn and make predictions, we performed chronic *in vivo* tetrode recordings in male rabbits across the entire expanse of the right pontine nuclei during 108 trial sessions during presentations of either a pure tone or eyelid conditioning sessions where a tone (CS) was paired with the stimulation of the periocular muscles (US) of the left eye. This allowed us to characterize a variety of response types (e.g. phasic, tonic, and feedback) in the pontine nuclei during the CS period both before and after the rabbits learned to make conditioned responses (CRs), measured as eyelid closure during the CS and preceding the US. While robust phasic responses may convey more information about the onset of the CS, tonic input to the cerebellum may be important for relaying the duration of the CS. Feedback responses, increases or decreases in pontine firing rate that occur after CR onset, have a putative role in chaining together movements or amplifying relevant stimulus inputs. This feedback is potentially driven via direct cerebellar output collaterals to the pontine nuclei or through thalamo-pontine projections receiving cerebellar feedback. These recorded pontine responses were also used as mossy fiber input in a simulation of the cerebellum in order to create a more biologically accurate model of the cerebellum. Since we can manipulate the simulation in ways that are not feasible in live animals, this allows us to ask questions about why certain aspects of the CS input may be important to the cerebellum for both learning and making accurate predictions. These methods allow us to characterize pontine responses during an auditory CS in order to ask questions about what input the cerebellum uses to learn and make predictions.

Disclosures: E.A. Kish: None. **M. Dodla:** None. **E.D. Delord:** None. **H.E. Halverson:** None. **M.D. Mauk:** None.

Poster

718. Cerebellar Networks and Functions

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Program#/Poster#: 718.04/QQ2

Topic: E.02. Cerebellum

Support: CIHR

Title: Selective encoding of unexpected head tilt by the central neurons takes into account the cerebellar computation output

Authors: *I. MACKROUS[1], J. CARRIOT[2], M. JAMALI[3], J. BROOKS[4], K. CULLEN[4]; [1]Physiol., McGill, Montreal, QC, Canada; [2]Physiol. and pharmacology, Univ. of Western Ontario, London, ON, Canada; [3]Harvard, Boston, MA; [4]Physiol., McGill Univ., Montreal, QC, Canada

Abstract: During daily activities, our sensory system is activated by self-generated and external events. We have previously shown that cerebellar output neurons and their targets neurons in the vestibular nuclei robustly encode passively applied motion in the horizontal plane, while they are attenuated during comparable self-generated head motion. However, natural head movements are not restricted to one plane and generate more complex vestibular stimuli because of the presence of the gravity. Specifically, sensory periphery does not provide an accurate representation of the gravity due to Einstein's equivalence. Therefore, we tested whether a neural representation of gravity is included in the internal model used to differentiate between passive and active motion. We first studied neuronal responses of cerebellar output neurons (rostral Fastigial nuclei, rFN) during passive tilts motion and found that neurons could be divided in three groups: translation-selective, tilt-selective and GIA selective cells. Sensitivity of the GiA selective cells was similar to translation and tilt. Tilt selective and translation selective cells showed a higher sensitivity for their respective preferred stimulation. We then recorded the responses of each neuron while monkeys made voluntary changes in head orientation by performing head tilt motion. Relative to their modulation in response to either passive translation or to passive tilt, neuronal responses to comparable self-generated movements were attenuated rFN (78%). This was consistent with prior characterizations for active translation in the horizontal plane showing a ~70% decrease in the neuronal modulation during active movements. Furthermore, because passive and active stimulation are often experienced simultaneously, we also tested whether neurons selectively encode sensory exafference under a condition where the monkeys were passively translated while generating simultaneous voluntary head tilt motion. When submitted to concomitant passive and active stimulation, both translation and GIA-selective cells responses provided a precise estimate of the passive motion. Such modulation was not expected for the tilt-selective cells as they do not encoded passive translation motion. Our findings demonstrated a sophisticated processing, where the internal model integrates not only an exogenous stimulus as shown previously, but also take into account the complex calculation of tilt-translation disambiguation.

Disclosures: I. Mackrous: None. **J. Carriot:** None. **M. Jamali:** None. **J. Brooks:** None. **K. Cullen:** None.

Poster

718. Cerebellar Networks and Functions

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Topic: E.02. Cerebellum

Support: Sloan Foundation

Whitehall Foundation

Title: Cerebellar complex spiking differentially encodes trial outcome in a voluntary forelimb task

Authors: J. HEFFLEY, A. MCKINNEY, *C. HULL;
Neurobiolgy Dept, Duke Univ., Durham, NC

Abstract: In the mammalian cerebellum, climbing fibers provide a powerful synaptic input to Purkinje cells that evokes regenerative dendritic calcium events called complex spikes. Complex spikes are generally thought to provide instructional signals that drive cerebellar-dependent motor learning. For many simple behaviors such as learned modifications to reflexes, climbing fibers have been shown to provide motor error signals. However, it is unclear whether such a model can explain more flexible behaviors where there are multiple sensory and motor signals necessary to drive learning, which themselves often need to be learned. Therefore, to test the role of complex spiking in motor learning, we have developed a forelimb-based motor learning paradigm for the head-fixed mouse that is amenable to calcium imaging and other recording methods, as well as optogenetic manipulation of neural activity. Here we address the question of what information complex spikes convey prior to learning, and how this information is distributed across large regions of the cerebellum during a motor behavior that can readily be modified by learning. By expressing GCaMP6f in cerebellar Purkinje cells, we studied complex spiking using both widefield imaging to visualize large regions of cerebellar cortex, and resonant scanning two-photon imaging to visualize complex spikes within individual Purkinje cells. Using these approaches, we find robust task-modulated complex spiking localized to lobule simplex, where Purkinje cells can drive ipsilateral arm movements. Contrary to a model where complex spikes signal motor errors, widefield imaging revealed larger amplitude calcium transients in response to successfully timed arm movements as compared with improperly timed movements. To test the underlying source of these differential responses, we imaged individual Purkinje cell dendrites at higher resolution using resonant scanning two-photon microscopy. These experiments revealed that well-timed movements produced both larger responses within individual dendrites, as well as a larger number of responsive dendrites across a local population of Purkinje cells. Thus, we find that task-modulated complex spiking is driven in a temporally specific manner that is linked to trial outcome, properties that are well suited to instruct motor

learning. However, our findings are not consistent with an error-based model of complex spike driven motor learning in our behavioral paradigm. Instead, our results suggest the hypothesis that complex spiking is more closely tied to the specific combination of sensorimotor inputs necessary to drive learning rather than to the valence of the inputs it encodes.

Disclosures: J. Heffley: None. **A. McKinney:** None. **C. Hull:** None.

Poster

718. Cerebellar Networks and Functions

Location: Halls B-H

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Topic: E.02. Cerebellum

Support: Wellcome trust grant RES/0165/7592/052

Title: The relationship between local field potentials in the primary motor cortex and the cerebellum during sleep

Authors: *W. XU[1], F. DE CARVALHO[2], A. JACKSON[2];
[1]Fac. of Med. Sci., [2]Newcastle Univ., Newcastle Upon Tyne, United Kingdom

Abstract: Studies of brain activity during sleep have hitherto largely concentrated on signals from the cerebrum. Despite the fact that both the cerebellum and sleep are heavily implicated in motor learning, very little is known either about cerebellar activity or its relationship to cerebral activity during different sleep stages. Using a wearable device we made recordings of local field potentials (LFPs) from the primary motor cortex (M1) and the cerebellum of the macaque during natural sleep in its home environment. We found that cerebellar and M1 LFP powers generally modulated together in time during sleep. We used modulation of M1 power in the delta band (0-4Hz) as a continuous measure of the periodicity of sleep. We took periods of relatively high delta power to represent slow-wave (stage 3) sleep and periods of relatively low delta power to represent non-slow wave sleep (stage 1, 2 and REM sleep). We find that power in higher frequency bands is modulated out of phase with delta power in both M1 and the cerebellum. Moreover we see a greater degree of coherence between M1 and cerebellar LFPs in these higher frequency bands during periods of relatively low M1 delta power, and vice versa. There is also a greater degree of periodicity in the modulation of powers of frequencies up to the low gamma range during sleep and the coherence between the two brain regions occurs in a different frequency ranges compared to waking. We conclude that the well-known cortical sleep states are associated with distinct patterns of activity in the cerebellum, and that behavioural state influences the interaction between motor cortex and cerebellum.

Disclosures: **W. Xu:** None. **F. De Carvalho:** None. **A. Jackson:** None.

Poster

718. Cerebellar Networks and Functions

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Topic: E.02. Cerebellum

Support: NIH Grant NS088567

Title: Amygdala inactivation impairs delay eyeblink conditioning with a visual conditioned stimulus.

Authors: *S. J. FARLEY, J. H. FREEMAN;
Dept of Psychological and Brain Sci., Univ. of Iowa, Iowa City, IA

Abstract: Amygdala modulation of cerebellar-mediated conditioned responses has recently been demonstrated (Farley et al, 2016, Siegel et al, 2015). A candidate mechanism is that amygdala output may modulate the conditioned stimulus (CS) pathway in delay eyeblink conditioning (dEBC). However, it is unknown if the amygdala modulation is specific to dEBC with an auditory CS. To address this, we trained adults rats in dEBC as previously reported (Farley et al, 2016), but used a white LED as the CS.Adults rats were bilaterally implanted with a cannulae directed at the amygdala central nucleus (CeA). Rats were divided into three groups: MUS (muscimol [GABA-A agonist]), SAL (saline), MUS-EXT (extensive training with muscimol). The MUS and SAL groups received muscimol (2mM, 0.2µl) or saline infusions 30 minutes prior to training during sessions 1-5. Training continued without infusions from session 6 to reaching criterion. Saline and muscimol retention tests were then given to all rats (order counter balanced). The CS was then switched to an auditory CS and rats reacquired dEBC. After reaching criterion, rats were given retention tests again with saline and muscimol infusions. For the MUS-EXT group, rats were trained for 16-sessions with CeA inactivation. Training continued without inactivation from session 17 until reaching criterion, after which they underwent a muscimol retention session.Acquisition of dEBC with a visual CS was severely impaired with CeA inactivation. There were no differences in the rate or magnitude of other eyeblink measures (unconditioned responses, startle responses, etc) during the initial LED training. The number of sessions to reach criterion without inactivation was the same between muscimol and saline animals across CS modalities. After rats in the MUS-EXT group reached criterion, their conditioned responses were impaired with a muscimol retention session and then CRs recovered to pre-retention levels the following session. These results show that CeA inactivation impairs the acquisition and retention of dEBC with either a visual or auditory CS.

Furthermore, despite some learning of the CS-US association with extensive CeA inactivation, amygdala output appears to have a persistent role in the acquisition and retention of dEBC. These results are consistent with previous findings that amygdala output to the pontine nucleus may strongly modulate CS information to the cerebellum.

Disclosures: **S.J. Farley:** None. **J.H. Freeman:** None.

Poster

718. Cerebellar Networks and Functions

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Topic: E.02. Cerebellum

Support: NIH Grant NS088567

Title: Amygdala modulates cerebellar learning through the conditioned stimulus pathway

Authors: ***L. J. CEMO**[1], S. J. FARLEY[2], J. H. FREEMAN[2];
[2]Psychological and Brain Sci., [1]Univ. of Iowa, Iowa City, IA

Abstract: Eyeblink conditioning (EBC) is an associative motor learning task requiring the cerebellum. Previous research has shown impaired acquisition and retention of EBC when the central amygdala (CeA) is inactivated with muscimol or bupivacaine in rats (Ng & Freeman, 2014; Farley et al., 2016). The medial CeA has a monosynaptic projection to the basilar pontine nuclei (PN), which relay conditioned stimulus (CS) information to the cerebellum via the middle cerebellar peduncle (MCP). Moreover, there is evidence that the CeA modulates CS-related activity in the PN (Taub & Mintz, 2010). However, the CeA has other projections that could influence the eyeblink conditioning circuitry. In this study, electrical stimulation of the MCP (50 Hz, 100 μA) was given as the CS during eyeblink conditioning in rats in order to bypass the amygdala projection to the CS pathway. The CeA was also pharmacologically inactivated during both acquisition and retention of EBC. If the CeA influences cerebellar learning through projections to the CS pathway, learning with MCP stimulation as the CS should be unaffected by CeA inactivation. On the other hand, if the CeA influences cerebellar learning through projections to the unconditioned stimulus, unconditioned response, or conditioned response pathways, CeA inactivation should at least partially impair EBC when using MCP stimulation as the CS. Acquisition and retention of EBC with MCP stimulation as the CS were completely unimpaired by CeA inactivation. These findings indicate that the amygdala exerts its modulatory effect on cerebellar learning exclusively through the CS pathway, possibly at the level of the PN.

Disclosures: **L.J. Cemo:** None. **S.J. Farley:** None. **J.H. Freeman:** None.

Poster

718. Cerebellar Networks and Functions

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Topic: E.02. Cerebellum

Support: Medical Research Council Career Development Award G1000512

National Research Foundation Singapore CRP grant

Title: Encoding of voluntary movement by cerebellar interneurons during active whisking

Authors: S. CHEN[1], G. J. AUGUSTINE[2], *P. T. CHADDERTON[1];
[1]Imperial Col. London, London, United Kingdom; [2]Lee Kong Chian Med. Sch., Nanyang Technological Univ., Singapore, Singapore

Abstract: The cerebellum is a major site of sensorimotor integration, but our understanding of how the circuit encodes associated sensory and motor information during behaviour remains limited. Here we use a well-defined model, the mouse vibrissae system, to study how sensorimotor signals are represented by the activity of individual neurons within the cerebellar cortex. We have recently shown that single Purkinje cells linearly encode whisker position via bidirectional firing rate changes (eLife 5: e10509). However, it is unclear how whisker information is processed before reaching Purkinje cells. We examined the contribution of synaptic inputs to this linear encoding scheme by directly recording from two types of upstream neurons, excitatory granule cells and inhibitory interneurons. In vivo patch clamp recordings were made from lobule Crus I in awake, head-restrained mice. Simultaneous high-speed videography and motion tracking allowed us to correlate spontaneous whisking behaviour with single neuron activity. We found that granule cells (n = 5/13 whole cell, 9/15 cell-attached) use high-frequency bursting activity to individually encode sparse and sharply tuned information about whisker set point. Granule cell populations thus provide to downstream neurons an overall linear excitatory drive correlated with set point change. In contrast, putative molecular layer interneurons (MLIs) exhibited bidirectional changes in firing rate during whisking (n = 34/43), with a large fraction displaying linear relationships (n = 19/43) between firing rate and whisker position in the same manner as Purkinje cells. Thus, feed-forward inhibitory signalling via MLIs functionally reverses the sign of whisker-related inputs received by Purkinje cells (and by other interneurons). Our results suggest that sparse and selective granule cell activity is integrated by

downstream neurons to provide a linear representation of volitional movement in MLIs and Purkinje cells.

Disclosures: **S. Chen:** None. **G.J. Augustine:** None. **P.T. Chadderton:** None.

Poster

718. Cerebellar Networks and Functions

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Topic: E.02. Cerebellum

Support: MEXT, Japan

Title: Temporal prediction signals in the cerebellar dentate nucleus are shaped by GABAergic inputs in behaving monkeys

Authors: ***A. UEMATSU**, M. TANAKA;
Hokkaido Univ. Sch. Med., Sapporo, Japan

Abstract: We previously found that neurons in the cerebellar dentate nucleus (DN) exhibited temporally-specific firing modulation for isochronously repetitive stimuli, which were proportional to the interstimulus interval (Ohmae et al., *J Neurosci,* 2013). To assess the relative contribution of the inputs from Purkinje cells (P-cells) and mossy fibers (MFs) to the firing modulation in the DN, we compared single neuronal activity before and during infusion of either $GABA_A$ receptor antagonist (gabazine, 1–4 mg/mL) or glutamate receptor antagonists (NBQX and CPP for AMPA and NMDA receptors, respectively; 0.5 mg/mL for each). Drugs were pressure-injected (~0.3 μL at 7–15 nL/min) during single neuron recordings using a homemade injectrode and a micropump. Three monkeys performed the "missing oddball" task, in which animals made a saccade in response to a single omission of repetitive visual stimuli that appeared at a fixed interstimulus interval. To accomplish this task, the animals had to predict the timing of each next stimulus in the sequence.
When gabazine was administrated, the magnitude of firing modulation for each repetitive stimulus significantly decreased (paired *t*-test, $n = 12$, $p < 0.05$), while the majority elevated the baseline firing rate ($n = 7/12$, $p < 0.05$). On the other hand, when NBQX+CPP compound was applied, the size of firing modulation remained unchanged ($n = 8$, $p = 0.96$), although most neurons reduced the baseline firing rate ($n = 5/8$, $p < 0.05$). Furthermore, the changes in baseline activity and those in the firing modulation tended to negatively correlate with each other in the gabazine experiments (Spearman's rank correlation, $r_s = –0.50$, $p = 0.10$), whereas they showed a trend of positive correlation in the NBQX+CPP experiments ($r_s = 0.52$, $p = 0.20$). We also found

that only a few neurons showed alteration of saccade-related neuronal activity during either drug infusion in the memory-guided saccade trials. These results suggest that the temporally-specific firing modulation in the DN might be generated by the inputs from the cerebellar cortex, although both the signals from MFs and P-cells may contribute to adjusting the level of baseline activity.

Disclosures: **A. Uematsu:** None. **M. Tanaka:** None.

Poster

718. Cerebellar Networks and Functions

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Topic: E.02. Cerebellum

Support: NS050808

NS079750

Title: Cerebellar modulation of the prefrontal cortex

Authors: ***A. SCHOTT**, K. KHODAKHAH;
Dominick P. Purpura Dept. of Neurosci., Albert Einstein Col. of Med., Bronx, NY

Abstract: The cerebellum is canonically known for its role in coordinating movement. However, evidence from several anatomical tracing studies suggests that some cerebellar projections terminate in non-motor areas. Recent work in our laboratory has revealed a functional monosynaptic connection between the deep cerebellar nuclei (DCN) and ventral tegmental area (VTA), which influences reward and motivational behaviors through dopamine signaling. The VTA is known to project directly to the prefrontal cortex (PFC), an area that has been implicated in complex cognitive processing. Here, we determined whether VTA cells that receive cerebellar input go on to project to cells in the PFC. For our anatomical tracing experiments, we used GFP-tagged H129, a strain of herpes simplex virus type 1 that trans-synaptically spreads predominantly in an anterograde direction. We found that in mice, injection of H129 into the DCN results in GFP-labeled cells in PFC. In order to assess the functional responses of PFC neurons to cerebellar activation, we performed extracellular *in vivo* recordings in awake, behaving mice. We found that optogenetic stimulation of Channelrhodopsin2-expressing cerebellar axons in VTA robustly modulates firing rates of both VTA and PFC neurons. We observed both excitatory and inhibitory prefrontal responses upon stimulation of cerebellar

fibers. These data suggest that the cerebellum communicates with the prefrontal cortex, and may have a substantial role in influencing cortical processing.

Disclosures: **A. Schott:** None. **K. Khodakhah:** None.

Poster

718. Cerebellar Networks and Functions

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Program#/Poster#: 718.12/QQ10

Topic: E.02. Cerebellum

Title: Cerebellar projections to VTA: reward and social roles

Authors: ***I. CARTA**[1], C. H. CHEN[2], S. DORIZAN[3], A. SCHOTT[1], K. KHODAKHAH[1]; [2]Neurosci., [1]Albert Einstein Col. of Med., Bronx, NY; [3]Neurosci., Northwestern Univ., Chicago, IL

Abstract: The cerebellum is an important structure for movement. However, some of its projections terminate in brain areas that are not related to movement. Cerebellar fibers are found throughout the midbrain, including the ventral tegmental area (VTA). The VTA, through dopaminergic signaling, participates in reward, motivation and socially related behaviors. We hypothesized that a cerebellar-VTA connection might be a substrate for cerebellar modulation of non-motor behaviors. To test this, we first examined the functional properties of this connection by expressing Channelrhodopsin2 (ChR2) in the deep cerebellar nuclei (DCN) and recording from the VTA either in vitro or in vivo while stimulating ChR2+ axons from the cerebellum. We found that responses in the VTA were common, and many of these cells were dopaminergic. In order to determine whether the cerebellar input to VTA is important to influence these complex behaviors we expressed either ChR2 or Archaerhodopsin (ArchT) in the DCN of mice, and bilaterally implanted optical fibers above VTA. We then performed behavioral testing to assess reward seeking and sociability. In a simplified self-stimulation test we found that optical stimulation of cerebellar terminals in VTA is sufficient to induce self-stimulation. Moreover, in the three chamber social task, we found that the natural preference of mice for social contexts is lowered if stimulation is offered as an alternative. To further determine whether the cerebellar inputs to VTA play a role in social behavior we silenced them using ArchT and indeed the social preference was decreased during the task. Finally, to test whether the cerebello-VTA pathway is naturally active during social behavior, we injected some mice with GCamp6 in DCN and implanted them with optic fibers in the VTA to detect calcium fluorescence. When we tested them in the three chamber social task, we observed increased calcium fluorescence while the mice were close to the social cue. Our data suggest that cerebellum might be an important

upstream structure that shapes VTA response to salient stimuli thereby influencing reward and social behavior.

Disclosures: **I. Carta:** None. **C.H. Chen:** None. **S. Dorizan:** None. **A. Schott:** None. **K. Khodakhah:** None.

Poster

718. Cerebellar Networks and Functions

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Topic: E.02. Cerebellum

Support: NIH R01 NS045193

Title: A center surround system for movement control in the deep cerebellar nuclei

Authors: ***S. A. HEINEY**[1], J. F. MEDINA[2];
[2]Dept. of Neurosci., [1]Baylor Col. of Med., Houston, TX

Abstract: We previously identified a small region in the anterior interpositus nucleus of the cerebellum that is essential for the expression of learned eyelid movements in mice, and reported that the responses of some neurons located in and around this “hotspot” are positively correlated with the eyelid kinematics on single trials. Here we analyzed how the same neurons respond during locomotion and report an unexpected feature of the response properties. Mice were head-fixed on top of a cylindrical treadmill and trained to blink in response to a light or vibrissal conditioned stimulus (CS) that was repeatedly paired with an airpuff to the eye. We then made single unit recordings in and around the eyeblink hotspot while the mice performed conditioned eyelid movements and walked freely on the treadmill. We found that many of the neurons that had excitatory responses during conditioned eyelid movements were inhibited during locomotion. Furthermore, the firing of nearby neurons was a mirror image: inhibition during conditioned eyelid movements and excitation during locomotion. This antagonistic relationship between neighboring neurons suggests that neurons within the deep cerebellar nuclei contain center surround motor fields analogous to the center surround receptive fields commonly seen in sensory systems.

Disclosures: **S.A. Heiney:** None. **J.F. Medina:** None.

Poster

718. Cerebellar Networks and Functions

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ARCS Foundation Award

Title: A hypothesized circuit mechanism underlying motor memory expression in cerebellum

Authors: *A. M. REEVES[1], T. S. OTIS[2];
[2]Neurobio., [1]UCLA, Los Angeles, CA

Abstract: The cerebellum has long been hypothesized to be involved in the storage and expression of motor memories. Recently, our lab showed that optogenetically conditioned motor memories are stored in the cortical and nuclear regions of the cerebellum and expressed via disinhibition-mediated bursts of activity in the cerebellar nuclei. The objective of this study was to determine the impact of preventing disinhibition of the cerebellar nuclei when cueing an optogenetically conditioned forelimb movement. By transiently increasing inhibition of cerebellar nuclei during high-speed video recordings of mice responding to a previously conditioned auditory cue, we demonstrated that preventing disinhibition of cerebellar nuclei prevents the expression of the learned forelimb movements. We conclude that disinhibition-mediated bursting of the cerebellar nuclei is necessary for expressing the motor memory and the subsequent forelimb movement. Furthermore, we suggest this mechanism could be relevant for the expression of other kinds of cerebellum-dependent motor memories.

Disclosures: **A.M. Reeves:** None. **T.S. Otis:** None.

Poster

718. Cerebellar Networks and Functions

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Topic: E.02. Cerebellum

Support: EY019258

Title: Spatial updating of saccades: What do the superior colliculus and cerebellum do?

Authors: *R. SOETEDJO;
Physiol. and Biophysics,, Univ. of Washington, Seattle, WA

Abstract: We are constantly moving, and these movements shift the visual representation of the target goal of an impending movement across the retina. The brain must re-compute the programming of the movement toward the goal to take into account the intervening movements so that our goal directed movement is accurate. Spatial updating of movements can be studied using saccades by exposing the subjects to double-step saccade task (DST). In this task, two sequential saccades are made in the dark following two flashes of a small target spot in different locations on a tangent screen. The first saccade disrupts the congruency between the vector of the retinal representation of the second target flash and the desired vector of the second saccade. Because there is no intervening saccade, in targeting saccade task the two vectors are congruent. In DST task, the superior colliculus (SC) is thought to send the desired saccade vector signal to brainstem burst generator to produce the second saccade. However, our data showed that the saccade-related bursts of neurons in the intermediate layer of the SC appeared to encode the initial vector of the retinal representation of the second target flash (retinotopic goal). The incongruity between the signal from the SC and the vector of the impending saccade requires a correction. For example, in a DST task in which the two target flashes appears at 8 and 16 degree eccentricities, the monkey must make two 8 degree saccades in the same direction. Saccade-related burst neurons located at 8 degree site burst for the first 8 degree saccade. They do not burst for the second 8 degree saccade, but neurons located at 16 degree site do. Therefore, the drive signal from the SC would be too large for producing 8 degree second saccade, and other neuronal structures must compensate for it. One candidate is the cerebellar caudal fastigial nucleus (cFN) because it projects directly to the burst generator. Neurons in cFN exhibit earlier burst for contraversive saccades and later burst for ipsiversive saccades. It is thought that the early burst is accelerating the contraversive saccades, and the late burst is decelerating ipsiversive saccades. Our recording from cFN neurons using DST task indicated that their burst timing and magnitude seem appropriate to compensate for the retinotopic goal signal from the SC during the execution of the second saccade. Taken together, these data argue that the oculomotor cerebellum may involve in computing the spatial updating of saccades.

Disclosures: R. Soetedjo: None.

Poster

718. Cerebellar Networks and Functions

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Topic: E.02. Cerebellum

Support: NIH Grant NS13742

Title: Comparing visuovestibular modulation of floccular interneurons and Purkinje cells; or, Where does the diversity go?

Authors: R. A. HENSBROEK, J. MARUTA, B. J. VAN BEUGEN, *J. I. SIMPSON; PHYSIOLOGY & NEUROSCIENCE, New York Univ. Sch. of Med., New York, NY

Abstract: "Neuronal machine" is a metaphor for the cerebellum, although knowledge of how the parts of the machine work together to provide an appropriate output is quite limited. This situation reflects the difficulty in identifying the activity of the various parts of the "machine", especially during an awake animal's behavior. Recently, we developed a decision tree using spontaneous firing patterns to identify classes of interneurons. The ability to distinguish among mossy fiber inputs, interneuron classes, and Purkinje cell outputs provides opportunities to study integration in cerebellar signal processing. We report here on the modulation of identified floccular Purkinje cells and interneurons in the awake rabbit responding to visuovestibular stimulation. Single-cell recordings were made in the flocculus of two awake rabbits that were sigmoidally oscillated about a vertical axis in the light. This stimulation allows determination of sensitivity to rotation direction and to the underlying movement kinematics. Earlier work in our laboratory showed that the vast majority of those rabbit floccular Purkinje cells whose complex spikes (CSs) were best modulated by visual image slip about the vertical axis responded in the dark with an increase in simple spike (SS) activity during contralateral head rotation and/or a decrease in SS activity during ipsilateral head rotation. (Responses with such a directional polarity are called type II. Responses with the opposite polarity are type I). Interestingly, floccular mossy fibers have been reported to be type I or type II in roughly equal numbers. In the present study, each interneuron class - unipolar brush cell, granule cell, Golgi cell, and basket/stellate cell - had both type I and type II members. Unipolar brush cells and granule cells had more type II than type I responsive cells (8 vs 4 and 12 vs 8, respectively), Golgi cells had more type I responsive cells (10 vs 3), while basket/stellate cells had nearly equal numbers of type I and type II responsive cells (6 vs 5). In marked contrast, in the present study the 16 Purkinje cells with CSs best responsive to visual slip about the vertical axis all had type II SS modulation. Taken together, the findings reveal that the roughly balanced presence of type I and type II input modulations is maintained on the interneurons, but is absent on the output Purkinje cells. The extent to which this loss of diversity was divided between moment-to-moment

computational processes and longer-term plastic processes is not yet known. In situations other than the one reported here, other SS modulation patterns may arise, drawing from the wide range of available interneuron diversity.

Disclosures: R.A. Hensbroek: None. **J. Maruta:** None. **B.J. van Beugen:** None. **J.I. Simpson:** None.

Poster

718. Cerebellar Networks and Functions

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 718.17/QQ15

Topic: E.02. Cerebellum

Support: JSPS KAKENHI No.24650224

Title: Intrinsic movement representation in the cerebrocerebellum

Authors: *T. ISHIKAWA[1], D. S. HOFFMAN[2,3], S. KAKEI[1];
[1]Tokyo Metropolitan Inst. of Med. Sci., Tokyo, Japan; [2]Dept. of Neurobio., [3]Cent. for the Neural Basis of Cognition, Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA

Abstract: In primates, the hemispheric part of the cerebellum is termed the cerebrocerebellum because it receives its primary input from the cerebral cortex via pontine nuclei. The ponto-cerebellar projection terminates as mossy fibers (MFs) in the granular layer of the cerebellar cortex. The MFs projecting to lobules IV-VI of the cerebrocerebellum receive predominant inputs from the primary motor cortex (M1) and premotor cortex (PM) in the frontal lobe (Kelly and Strick 2003). The output of this part of the cerebrocerebellum is generated in the dentate nucleus (DN) and sent back to M1 or PM via thalamus. Because of this anatomical structure, examination of activity patterns of the MFs and DN cells during a movement should provide important clues for understanding the functional role of the cerebrocerebellum in control of voluntary movement.
We recorded unit activity of MFs and DN cells in 2 monkeys during step-tracking movements of the wrist joint in two forearm postures: full pronation (Pro) and full supination (Sup). We found 52 MFs and 51 DN cells with significant task-related activity in both forearm postures. Most of these (n=49 MFs and n=48 DN cells) had clear somatosensory receptive fields in the distal part of the ipsilateral arm. For each cell, we calculated a task-related preferred direction (PD) before movement onset and determined the change in PD from Pro to Sup postures. More than half of the MFs (n=27) and DN cells (n=27) showed directional tuning in both forearm postures and had a significant change in PD (40-120 deg) between postures. This change in PD was comparable to

that of EMG activity of task-related forearm muscles (average 65 and 100 deg in the two monkeys) and also matched that of "intrinsic-like" M1 cells reported in a previous study (Kakei et al. 1999). Furthermore, the timing of the modulations in MFs and DN cells preceded movement onset (average of 83 and 77 msec, respectively). Taken together, our results are consistent with the view that MFs projecting to lobules IV-VI of the cerebrocerebellum transmit an efference copy of motor cortical activity prior to movement onset. The timing of DN output to M1 and PM is early enough to provide a prediction of the sensory outcome of the planned movement prior to any sensory feedback from the actual movement. Thus, our results indicate that the cerebrocerebellum contains a forward model directed to frontal lobe motor areas to improve the precision of motor control.

Disclosures: **T. Ishikawa:** None. **D.S. Hoffman:** None. **S. Kakei:** None.

Poster

718. Cerebellar Networks and Functions

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Program#/Poster#: 718.18/QQ16

Topic: E.02. Cerebellum

Support: NIH Grant R37 NS39395

Title: Firing of Purkinje cells and neurons of the cerebellar nuclei during free and optogenetically perturbed locomotion in awake mice

Authors: *R. SARNAIK, I. M. RAMAN;
Dept. of Neurobio., Northwestern Univ., Evanston, IL

Abstract: Purkinje (Pkj) cells change their firing rates during movements and modulate the activity of target cells in the cerebellar nuclei (CbN) via convergent, GABAergic projections. Large CbN cells generate cerebellar output that can facilitate coordinated movements or correct errors. To test how the firing rates of Pkj and CbN cells change during rest and locomotion, and to examine how Pkj firing patterns affect CbN cells, we made loose cell-attached recordings in head-fixed mice running ad lib on a cylindrical treadmill. The position of the ipsilateral hindpaw was monitored so that firing could be examined relative to the step cycle. Mice expressed channelrhodopsin (ChR2) in Purkinje cells (L7-cre x Ai27D), and illumination was applied through an optical fiber in the patch pipette. Optogenetic stimulation of Pkj cells near the recording pipette was used (1) to activate Pkj or inhibit CbN cells and so confirm the identities of neurons, (2) to evoke light-induced discontinuities in ipsilateral hindlimb movement and so verify that cells were in an ipsilateral hindpaw-specific region in lobule VI (Pkj) and in the

anterior interpositus (CbN), and (3) to manipulate Pkj cell firing patterns. When mice were stationary, the firing rates of both Pkj and CbN cells were similar, and, when mice ran, the mean rates of both groups increased (Pkj: rest 69 ± 7, run 109 ± 9, n = 26; CbN: rest 71 ± 5, run 92 ± 6 sp/s, n = 27). During running, most Pkj and CbN cells modulated their firing consistently at distinct phases of the step cycle, usually regardless of the stride length. Plots of instantaneous spike rates relative to the step cycle showed that firing could be (I) in phase, (II) 90 degrees shifted, with rates rising on the stance, (III) out of phase, (IV) 270 degrees shifted, with rates rising on the swing, (V) doubly peaked, with rises on the stance and swing, (VI) doubly peaked, with decreases on the stance and swing, or (VII) unmodulated. Pkj and CbN cells were found in all categories, with the largest groups of Pkj cells in classes II (n = 6/26) and V (n = 11/26) and the largest groups of CbN cells in classes I (n = 9/32) and II (n = 9/32). Optical stimulation of Pkj cells could perturb ipsilateral hindpaw movement in running mice or initiate running in stationary mice. In each cell, the light stimulus was varied, including steps (1-2 s) of different intensities and trains at several rates (1-ms pulses, 20-225 Hz). Even when mean firing rates of Pkj cells were matched, movement was more likely to be altered by light steps than by light trains, especially with Pkj firing rates <100 Hz. These data suggest that cerebellar output can vary with temporal patterns of Pkj cell activity.

Disclosures: **R. Sarnaik:** None. **I.M. Raman:** None.

Poster

718. Cerebellar Networks and Functions

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Title: The ghost in the machine: long range cerebellar signals

Authors: *L. S. POPA, Mr, M. L. STRENG, T. J. EBNER;
Dept. of Neurosci., Univ. of Minnesota, Minneapolis, MN

Abstract: The cerebellum is essential for precise and skillful motor behaviors. The time frame for expression of cerebellar function in motor control is generally assumed to be relatively short, close to real time. However, increasing evidence for cerebellar involvement in sequencing and working memory argues that cerebellar function manifests over extended time periods in both motor and cognitive domains.
In this study we examine the time frame of the cerebellar cortex signaling. Inter-temporal regression analysis of simple spikes (SS) firing recorded from 183 Purkinje cells during pseudo-random tracking reveals that modulation with kinematics (position, velocity and speed) and performance errors (position and radial errors) persists up to 2 s before and after behavior. The significance of the SS modulation for each behavioral parameter was assessed against regressions obtained with trial shuffled data that decoupled SS firing from behavior while preserving data statistics. Importantly, the long range signals in the SS firing were abolished in the trial shuffled data. For each behavioral parameter we divided the ± 2000 msec window of interest into eight epochs of 500 msec. The presence of signals was based on the largest significant local maxima of the R^2 temporal profile in each epoch. Position, velocity and position errors have the most frequent and strongest long-range predictive and feedback modulations, with less common, weaker long-term correlations for speed and radial error. Autocorrelations of the behavioral parameters show no secondary peaks that could explain the long range correlations. Furthermore, cross-correlations between behavioral parameters reveal only a few pairs with strong interactions that cannot fully explain the SS encoding of those parameters. Position, velocity and position errors can be decoded from the population SS firing with remarkable accuracy for even the longest predictive (-2000 to -1500 msec) and feedback epochs (1500 to 2000 msec). In the -500 to 0 and 0 to 500 msec epochs, decoding for all behavioral parameters is almost perfect.
We conclude that Purkinje cells SS discharge includes not only short-range predictive and feedback signals but also long-range activity over ± 2 s related to working memory and expectations of future behavior. Such long-range signals could provide the neural substrate underlying cerebellar involvement in working memory and sequencing. These results suggest the cerebellum evaluates the consequences of motor commands over a time frame consistent with the widespread 3-4 sec integration window characterizing motor, sensory and cognitive processes, considered to represent the "subjective present".

Disclosures: **L.S. Popa:** None. **M.L. Streng:** None. **T.J. Ebner:** None.

Poster

718. Cerebellar Networks and Functions

Location: Halls B-H

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Program#/Poster#: 718.20/QQ18

Topic: E.02. Cerebellum

Support: NIH R01 DC004154

NSF DGE-114747

NIH P30 NS069375

Title: The fine temporal structure of neural spike trains impacts motor behavior

Authors: *H. L. PAYNE[1], R. LI[1], C. C. GUO[2], J. L. RAYMOND[1];
[1]Stanford Univ., Stanford, CA; [2]QIMR Berghofer, Brisbane, Australia

Abstract: Neural spike trains can be characterized by both their overall firing rate and precise temporal patterning. Whereas firing rate is widely assumed to affect the activity of downstream neurons, the functional significance of the fine temporal structure of neural spike trains has been more difficult to test. An understanding of this functional impact may be especially relevant in designing and interpreting stimulation experiments, which typically use perfectly regular stimulus trains, although neural activity *in vivo* is often highly irregular.
We leveraged the experimental advantages of the oculomotor cerebellum to test the effects of the fine temporal structure of spike trains in intact animals. Optogenetic stimulation of Purkinje cells, the output neurons of the cerebellar flocculus, drove smooth eye movements time-locked to the stimulus. We investigated the interaction between two parameters: the irregularity and mean rate of Purkinje cell stimulation. Irregular activation patterns drove significantly smaller mean eye movements than the corresponding regular pattern at the same underlying rate. A biophysical model of the Purkinje cells' target neurons, modified from Luthman et al. (2011), was used to investigate how the irregularity of stimulus-driven, inhibitory synaptic input from Purkinje cells interacts with ongoing spontaneous input to control downstream pre-motor targets.
Finally, we assessed the impact of firing rate irregularity in the more natural context of eye movements driven by sensory stimuli. Recordings from Purkinje cells during oculomotor behaviors in both mice and monkeys revealed that the irregularity of spiking, as well as the spike rate, varied rapidly during behavior. Naturalistic sequences of concurrent changes in firing rate and irregularity were then played back optogenetically to Purkinje cells *in vivo.* The results provide causal evidence that the changes in spiking irregularity driven by sensory stimuli can serve to amplify the impact of firing rate changes on motor output.

Disclosures: **H.L. Payne:** None. **R. Li:** None. **C.C. Guo:** None. **J.L. Raymond:** None.

Poster

718. Cerebellar Networks and Functions

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 718.21/QQ19

Topic: E.02. Cerebellum

Title: Cross correlation and coherence study of cerebellar and cerebral cortical responses to tDCS using an *In vivo* approach

Authors: S. GEEVARUGHESE, J. H. YOO, *H. LU;
PCOM - Georgia Campus, Suwanee, GA

Abstract: The use of transcranial direct current stimulation (tDCS) as a potential therapeutic treatment for movement disorders associated with cerebellum has shown promise according to early studies. Our previous studies on the effects of tDCS to the cerebellum using rats suggested there were changes in Purkinje cell firing rate and local field potentials (LFPs). In our current investigation, dual recordings from the cerebellar and motor cortices were conducted on six Sprague-Dawley rats. A total of 16 Purkinje cells were isolated *in vivo* along with LFP recordings in both cerebellar and cerebral cortices with anodal tDCS at two different intensities: 100 μA and 200 μA. Both types of stimulation generated an overall decrease in Purkinje cell firing rate by 13%, which is in contrast to previous studies showing an increase. This indicates that the change in firing rate of the Purkinje cells is variable. Power spectrum analysis of LFPs in the motor cortex by tDCS revealed frequency changes in 13 out of 16 recordings. These changes are associated with an activity peaked at a frequency ranged from 85-100 Hz. In 3 cases, there is an immediate increase in amplitude for low frequencies following stimulation. The results support previous studies that electrophysiological changes in the motor cortex are associated with the activity changes in cerebellar cortex. We further studied the tDCS effects on cerebello-cerebral cortical relationship using cross correlation and coherence methods. Analysis shows a cross correlation ranging from 0.2-0.5 after normalization in 15 of 16 recordings. Further analysis on cross correlation lags indicates that the signal from the motor cortex precedes that of the cerebellar cortex although a variable change by tDCS is observed. Coherence analysis using magnitude squared coherence estimation shows correlation in two frequency ranges: 5-25 Hz and 85-100 Hz. The higher frequency range appeared in the power spectrum analysis for LFPs in both cortical regions. Future studies will include a computational modeling method to study the tDCS effects on a single Purkinje cell to help explain the bidirectional changes in firing rate and its involvement in the changes of cerebello-cerebral correlation.

Disclosures: **S. Geevarughese:** None. **J.H. Yoo:** None. **H. Lu:** A. Employment/Salary (full or part-time): PCOM - GA campus.

Poster

718. Cerebellar Networks and Functions

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 718.22/QQ20

Topic: E.02. Cerebellum

Title: A cerebellar commissure carries projections of the vestibular ganglia and the vestibular nuclear complex

Authors: D. T. DALY[1], *M. ARIEL[2];
[1]Ctr. for Anatom. Sci. and Educ., [2]Pharmacol. & Physiol., St. Louis Univ. Sch. of Med., Saint Louis, MO

Abstract: At SfN '15, we described a transverse cerebellar commissure of thick myelinated axons within the Purkinje Cell Layer (PCL) in the pond turtle *Trachemys Scripta*. This commissure connects the lateral edge of the cerebellar cortex (Cb) on one side to that on the other. Electrical stimulation of one eighth cranial nerve (cnVIII) evokes a rapid response in the contralateral edge of the Cb (Brown et al, J Neurophysiol 2011). The present work confirms the vestibular role of this commissure in mediating connections to vestibular afferents of the vestibular ganglia (VG) and to neurons of the vestibular nuclear complex (VNC). Intact brains of anesthetized turtles, with attached temporal bones, were prepared for pathway tracing from fluorescent dextrans (in vitro) placed in lateral Cb or carbocyanine dyes (fixed tissue) placed in cnVIII. Following fixation (4% para), decalcification (0.1M EDTA), and embedding in 3% gelatin, 30-μm sections were visualized with confocal microscopy. Commissural fibers were again seen in the PCL. Dextran filled somata were observed in both the ipsilateral and contralateral VG. This finding supports the lateral Cb's common name 'vestibulocerebellum', providing direct evidence of a vestibular input rather than only a general nVIII input. Somata observed bilaterally within the VG have bipolar morphology, measuring up to 18 μm wide and 29 μm long. Neurons were also observed bilaterally in VNC with multipolar soma diameters up to 20 μm. Additional experiments left the neural structures intact in fixed tissue except for a midsagittal cut through the brainstem below the Cb, from the obex to the midbrain. Then, one cnVIII was severed and DiI crystals were placed into that proximal nerve stump. That preparation was incubated for 5 weeks at 35°C before analysis. DiI-labeled cell bodies were observed bilaterally in each VNC at the same level as those labeled with dextrans and had similar morphology. The presence of labeled VNC somata contralateral to the dye placement, in a brainstem severed along the midline, indicates that the Cb provides a commissural path that is distinct from commissural fibers within the ventral brainstem. When observed in tangential sections, this commissure is located in the middle third of the Cb's rostral-caudal axis. Both DiI transport from cnVIII to the contralateral VNC after severing the brainstem mid-line, and the filling of contralateral VG cells from the lateral Cb, further elucidate the nature of the cerebellar

commissure and its anatomical relationship to the vestibular system. This thick, myelinated vestibular commissure traveling within the PCL may be integral in the bilateral processing of head movements.

Disclosures: D.T. Daly: None. **M. Ariel:** None.

Poster

718. Cerebellar Networks and Functions

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Topic: E.02. Cerebellum

Support: NIH Grant R01NS045702

NIH Grant P30HD040677

Title: Cerebellar learning deficits in a mouse model of premature birth injury

Authors: *A. SATHYANESAN, V. GALLO;
Ctr. for Neurosci. Res., Children's Natl. Med. Ctr., Washington, DC

Abstract: Premature births constitute a rapidly rising percentage of live births in the US. Annually ~65,000 preterm infants are born ‘very low birth weight’ or ‘VLBW’ (<1500 g; 32 weeks gestation), making them 40-100 times more likely to develop disruptive motor disorders compared to regular birth weight infants. Biomedical studies have established perinatal hypoxia as a major cause of grey and white matter abnormalities in the CNS of VLBW infants, with the cerebellum being particularly vulnerable to hypoxic injury. Previous work from our laboratory has shown that GABAergic signaling is significantly reduced in the white matter of the cerebellum in an established mouse model of perinatal hypoxic injury [Zonouzi, M. et al. GABAergic regulation of cerebellar NG2 cell development is altered in perinatal white matter injury. *Nature Neuroscience*, 2015]. Quite strikingly, the cerebellar cortex is also affected post-hypoxic injury (Hx). Hx results in a drastic reduction in number of molecular layer interneurons, and dramatically altered Purkinje cell arborization compared to normoxic controls (Nx). However, whether this cellular alteration in the cerebellar cortex due to hypoxic insult results in a difference in motor behavior remains unexplored. Using an automated behavioral apparatus - the Erasmus Ladder - to monitor motor performance and learning we have studied cerebellar behavior in Hx mice. This horizontally-oriented computerized ladder allows us to measure stepping patterns using pressure-sensitive rungs. Our results show that compared to normoxic controls, P25 Hx mice display profound deficits in both motor performance - measured as

percentage of missteps on the ladder, as well as conditioned motor learning - measured as adaptability to adjust stepping patterns to avoid a computer-controlled obstacle preceded by a warning tone. This deficit in motor performance and learning is present even in naïve P45 Hx mice, albeit to a lesser extent than P25 Hx deficits. Further, stepping pattern data indicate alterations in locomotor strategy in Hx mice. Finally, misstep percentage is partially rescued after pharmacological intervention with a GABA reuptake inhibitor in P25 Hx animals. Thus, our data indicates a long-term miscoordination phenotype characterized by motor malperformance, as well as adaptive cerebellar-learning deficits in a mouse model of premature birth injury.

Disclosures: A. Sathyanesan: None. **V. Gallo:** None.

Poster

718. Cerebellar Networks and Functions

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 718.24/RR2

Topic: C.07. Ischemia

Support: NIH NINDS K01 NS086969-01

Title: A novel mouse model of cerebellar stroke

Authors: M. MORENO, R. EMS, J. YONCHEK, *N. QUILLINAN;
Anesthesiol., Univ. of Colorado, Aurora, CO

Abstract: Objectives: Patients who have suffered cerebellar stroke present with neurological impairments such as motor coordination, motor learning deficits, vertigo and acute hearing loss. Cognitive-affective impairments have also been reported in these patients, suggesting an important role for the cerebellum in non-motor function. There are no animal models of cerebellar stroke currently available to investigate mechanisms of injury and repair. Therefore we have developed a mouse photo-thrombotic model of cerebellar stroke.
Methods: Adult male mice (C57Blk6, 8-12 week) were subjected to an intraperitoneal administration of Rose Bengal (150 ug/g), a light-sensitive dye that reacts with light and generates thrombosis by causing platelet aggregation. The injection was followed by a 15 minute exposure to a cold white light source. Sham controls were exposed to the light but were not injected with Rose Bengal. Stereological analysis of hematoxylin and eosin (H&E) staining was performed at 1 and 7 days after cerebellar stroke. Evans blue extravasation was used to interrogate blood brain barrier (BBB) integrity and immunohistochemistry was used to assess glial responses and formation of glial scar in the infarcted area. Neurobehavioral testing

including cylinder task, footprint analysis and contextual fear conditioning, were performed 7 days after cerebellar stroke.
Results: A cerebellar infarct and histological injury to cerebellar cortex was visible at both 24 hours and 7 day time-points after stroke onset. Infarct was localized to the intermediate zone of cerebellar cortex and a glial scar was present 7 days after stroke. Evans Blue dye was present in cerebellar stroke mice compared to controls, suggesting BBB disruption. Stroked animals showed changes in paw preference in the cylinder task suggesting a limb impairment. Footprint analysis showed reduced stride length and increased displacement between paws, indicating gait abnormality. Surprisingly, contextual fear conditioning testing showed impaired memory acquisition in cerebellar stroke animals compared to controls.
Conclusions: We have developed a reproducible model for cerebellar stroke that causes motor deficits. These outcomes are consistent with injury in the intermediate and lateral zones of the cerebellum. Also, we have been able to observe non-motor deficits that correlate with clinical studies of patients who have suffered a cerebellar stroke. This novel model will allow us to test neuroprotective and neuro-restorative strategies to improve functional outcome after cerebellar stroke.

Disclosures: **M. Moreno:** None. **R. Ems:** None. **J. Yonchek:** None. **N. Quillinan:** None.

Poster

719. Cerebellum: Anatomy, Cytoarchitecture, and Connectivity

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 719.01/DP07 (Dynamic Poster)

Topic: E.02. Cerebellum

Title: Purkinje cell heterogeneity in the anterior and posterior cerebellar lobule

Authors: *H. NEDELESCU[1], M. ABDELHACK[2], A. T. PRITCHARD[3];
[1]Dept. of Systems Physiol., Tokyo Med. and Dent. Univ., Tokyo, Japan; [2]Grad. Sch. of Informatics, Kyoto Univ., Kyoto, Japan; [3]Independent Scholar, Manchester, United Kingdom

Abstract: In the cerebellum, regional differences in cytoarchitecture could affect local connectivity and physiology, with possible consequences for neuronal computation. Analyses of Purkinje cells - which are functionally critical as they provide the sole output - have suggested that the cerebellar cortex is not uniform in structure as traditionally assumed. However, there have been insufficient numbers of reconstructed cells to resolve systematic differences in morphology between different cerebellar regions. To address this question, we compared Purkinje cell architecture in an anterior and a posterior lobule of the cerebellar cortex. Using Neurolucida 360 with confocal stacks from mice expressing green fluorescent protein selectively

in Purkinje cells, we reconstructed dendritic arbors of Purkinje cells residing in the bank of their respective lobules, then analyzed their structure and organization. We observed that in the posterior lobule, half of the reconstructed Purkinje cells had two primary dendrites emanating from their soma, in both young and adult mice. By contrast, only 9 out of 38 young and 5 out of 44 adult Purkinje cells showed this characteristic in the anterior lobule. Furthermore, our analysis revealed that Purkinje cells in the anterior lobule exhibited slanted dendritic arbors, oriented more towards the apex of the lobule than those found in the posterior lobule. This difference only appeared in the mature cerebellum, after the dendritic arbors had attained their definitive morphologies. These differences in Purkinje cell morphology support a microenvironment which could facilitate differential patterns of afferent input, and will need to be considered in future studies of cerebellar neural computation.

Disclosures: **H. Nedelescu:** None. **M. Abdelhack:** None. **A.T. Pritchard:** None.

Poster

719. Cerebellum: Anatomy, Cytoarchitecture, and Connectivity

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 719.02/RR3

Topic: E.02. Cerebellum

Support: AE Espino

PAPIIT UNAM Grant 206616

CONACYT Grant 220224

Title: Changes induced by hypoxic preconditioning in $GFAP^+$ cells from the roof of the fourth ventricle

Authors: M. BECERRA-GONZÁLEZ, R. DURAIRAJ VARMAN, *A. MARTINEZ-TORRES;
INB-UNAM, Queretaro, Mexico

Abstract: Introduction. Cerebellum is known for its role in motor control. It harbors a uniform cytoarchitecture composed of neuronal (molecular, granular and Purkinje neurons) and glial (astrocytes and Bergmann glia) lineage. We recently reported the presence of a cellular niche composed of diverse cell phenotypes located on the roof of the fourth ventricle that spans lobes I and X. The niche includes glial fibrillary acidic protein ($GFAP^+$), and $nestin^+$ cells distributed along the antero-posterior axis. Niches of $GFAP^+$ and $nestin^+$ cells in the lateral ventricles are well known neuronal or glial precursors that have the ability to respond upon hypoxic

preconditioning (HP). Yet, little cell proliferation has been demonstrated in cerebellum.
Aims. To evaluate the distribution of $GFAP^+$ cells on the roof of the fourth ventricle, and to determine whether they respond to hypoxic preconditioning (HP), a condition that promotes cell proliferation.
Methods. 25 days-old transgenic male mice expressing the enhanced green fluorescent protein under the GFAP promoter (GFAP-eGFP) underwent a three-cycle session of oxygen deprivation. Brains were collected at days 1 to 7 after HP. Distribution of the glial component was assessed by standard histological techniques and confocal microscopy, and western blot analyses evaluated changes in the levels of GFAP expression after HP.
Results. The organization of Bergmann glia cells was altered, somas were displaced and processes disorganized. Within the array of $GFAP^+$ cells of the roof of the fourth ventricle the expression of eGFP decreased after HP, but rebounded by day 7. This was confirmed by means of Western blot using antibodies against GFAP.
Conclusions. HP induces morphological changes in the Bergmann glial cells. The changes are minimal after seven days. HP reduces the expression levels of eGFP, which returns to normal levels after seven days. Consistently, expression of GFAP is also reduced by HP and returns to normal levels within the same period of time. In general, HP induces changes in the glial cells from cerebellum that induces a response to low levels of oxygen due to HP. Whether the $GFAP^+$ cells proliferate and differentiate as well as the molecular mechanisms of the cellular response under HP conditioning remain to be elucidated.

Disclosures: **M. Becerra-González:** None. **R. Durairaj Varman:** None. **A. Martinez-Torres:** None.

Poster

719. Cerebellum: Anatomy, Cytoarchitecture, and Connectivity

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Program#/Poster#: 719.03/RR4

Topic: E.02. Cerebellum

Support: NIH R37-NS39395

Title: Synaptic excitation by climbing fiber collaterals in the deep cerebellar nuclei of juvenile and adult mice

Authors: M. NAJAC, *I. M. RAMAN;
Neurobio., Northwestern Univ., Evanston, IL

Abstract: Neurons in the inferior olive (IO) give rise to climbing fibers (CFs) that excite Purkinje cells and send collaterals (CFCs) to the cerebellar nuclei (CbN). Because *in vivo* and *in vitro* data suggest that CFC-CbN excitation is weak in adult mice, we examined CFC input to large CbN cells over development. We expressed channelrhodopsin in CFs with viral injections to the IO of P1 or P21 mice and recorded from large CbN cells in acute cerebellar slices from P12-P22 (juvenile, "*juv*") and P44-P50 ("*adult*") mice. CFCs were activated by 1- or 2-ms blue light pulses. CFC stimulation transiently increased CbN cell instantaneous firing rate (*juv*: from 46 ± 12 to 241 ± 66 Hz, n=4; *adult*: from 88 ± 8 to 109 ± 14 Hz, n=5; cell attached mode). In 6/9 cells (*juv*: 3/4, *adult*: 3/5), responses were well-timed, with a 0.8 ± 0.1 spike probability in a 2-ms window after the pulse. EPSC amplitude at -70 mV was -685 ± 118 pA in juveniles (n=38), but only -166 ± 20 pA in adults (n=16), suggesting that synaptic strength decreases with age. EPSCs had short but variable latencies (*juv*: 2.2 ± 0.1 ms, jitter 0.2 ± 0.03 ms; *adult*: 2.4 ± 0.1 ms, jitter 0.3 ± 0.06 ms). 5-Hz trains of EPSCs depressed strongly, indicating a high release probability (*juv*: $EPSC_{2/1}$ 0.50 ± 0.03, $EPSC_{5/1}$ 0.36 ± 0.03, n=30; *adult*: $EPSC_{2/1}$ 0.32 ± 0.07, $EPSC_{5/1}$ 0.22 ± 0.04, n=10). Most cells (*juv*: n=30/33, *adult*: 13/16) showed composite EPSCs on most trials with 2-6 detectable peaks within 1-6 ms. We tested whether peakiness arose from burst firing in CFCs, polysynaptic activation, firing in multiple convergent CFCs, or asynchronous release from one axon. In loose-cell-attached recordings from IO axons, light evoked a variable-latency spike (0.9 ± 0.2 ms, jitter 0.07 ± 0.02 ms, n=26), but only 5/26 of axons fired bursts (2-4 spikes). Consistent with monosynaptic connections, NMDAR EPSCs were evident in CbN cells at positive voltages even with AMPARs and $GABA_A$Rs blocked (n=4/4). Peakiness remained with reduced activation of feed-forward pathways (low [Ca]out, n=5; low DNQX, n=5), making a polysynaptic origin unlikely. In juveniles, raising Ca from 1.5 to 3 mM affected neither the amplitude, charge, shape, nor depression of EPSCs, suggesting that release from CFCs was already maximal (n=7). Conversely, reducing Ca to 0.5 mM decreased EPSC amplitude and charge to 43 ± 5 % and modestly reduced depression ($EPSC_{2/1}$ 0.73 ± 0.06, $EPSC_{5/1}$ 0.43 ± 0.08, n=5) while leaving EPSC shape, i.e., the temporal structure of release, unaffected. Thus, the excitatory strength of CFCs onto large CbN cells decreases with age, but throughout development, CFCs evoke peaky EPSCs, likely resulting from innervation by multiple fibers and/or release asynchrony in individual afferents.

Disclosures: **M. Najac:** None. **I.M. Raman:** None.

Poster

719. Cerebellum: Anatomy, Cytoarchitecture, and Connectivity

Location: Halls B-H

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Topic: E.02. Cerebellum

Support: National Institutes of Health Grant NS083984 (J.M.C.).

Max Planck Society

Max Planck Florida Institute (MPFI) for Neuroscience

Title: Genetic targeting of cerebellar molecular layer interneurons using the c-kit promoter

Authors: *S. B. AMAT, M. A. GAFFIELD, M. J. M. ROWAN, A. BONNAN, C. KIKUCHI, H. TANIGUCHI, J. M. CHRISTIE;
Max Planck Florida Inst. For Neurosci., Jupiter, FL

Abstract: Genetic-targeting strategies have proven useful in understanding the role of individual neuron types in circuit function and behavior. In the cerebellum, Purkinje cells (PCs) are the sole output neuron type and receive inhibition from molecular layer interneurons (MLIs). Using fate mapping to distinguish MLIs from PCs has been challenging because both cell types are GABAergic. Without the means to target MLIs, the role of molecular layer inhibition in motor control and adaptive learning is enigmatic. To address this challenge, we made a knock-in mouse to express Cre recombinase (c-kit-IRES-Cre) in cerebellar MLIs under control of the c-kit oncogene allele. In mature mice, we achieved high-level transduction of MLIs using adeno-associated viruses (AAV) with Cre-dependent fluorescent proteins and, notably, had no PC labeling. We functionally validated this result by virally expressing either optogenetic or chemogenetic actuators in c-kit-cre mice. We observed a high-degree of specificity in manipulating MLI activity. In conclusion, the c-kit-IRES-Cre mouse line proved to be a flexible and reliable tool to target MLIs and will allow the interrogation of molecular layer inhibition in cerebellar circuits using genetically-encoded effectors and reporters of activity.

Disclosures: S.B. Amat: None. **M.A. Gaffield:** None. **M.J.M. Rowan:** None. **A. Bonnan:** None. **C. Kikuchi:** None. **H. Taniguchi:** None. **J.M. Christie:** None.

Poster

719. Cerebellum: Anatomy, Cytoarchitecture, and Connectivity

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Topic: E.02. Cerebellum

Support: NWO-ALW 824.02.001

Title: The basal interstitial nucleus provides inhibitory afferent input to the cerebellar flocculus

Authors: *D. JAARSMA[1], F. BLOT[2], M. SCHONEWILLE[2], Z. GAO[2], T. J. H. RUIGROK[3], C. I. DE ZEEUW[2,4];
[1]Dept. Neurosci. Erasmus MC, Rotterdam, Netherlands; [3]Neurosci., [2]Erasmus MC, Rotterdam, Netherlands; [4]Netherlands Inst. for Neuroscience,, Amsterdam, Netherlands

Abstract: *BIN neurons (arrows) and Golgi cells (arrow heads) in the flocculus of a GlyT2-EGFP mouse.*
The cerebellar cortex is known for its simple and well characterized neuronal circuitry, consisting of two main excitatory afferent systems, the climbing fibers and mossy fibers that converge on a single output neuron, the Purkinje cells. In addition, the cerebello-cortical circuitry contains granule cells that transfer mossy fiber input to Purkinje cells, and at least 4 classes of inhibitory interneurons and one class of excitatory interneuron. Here we show that the flocculus, a part of the vestibulocerebellum, receives a unique additional afferent input: We show that neurons of the basal interstitial nucleus (BIN), a hitherto neglected cerebellar nucleus originally identified by Langer in macaque cerebellum (J Comp Neurol. 1985, 235:26), provides widespread GABAergic/glycinergic afferents to the glomeruli of the floccular granule cell layer. We mapped the BIN in macaque, man, rabbit and rodent cerebellum using retrograde tracing (rabbit, rat, mouse), by immunostaining for ChAT (macaque and man), Gad65/67 (all species) and the muscarinic M2 receptor (all species), and by using a GlyT2-EGFP reporter mouse line. In macacque, man and rabbit BIN neurons are mainly distributed in the white matter between the lateral cerebellar nucleus and the flocculus, while in rodent they are concentrated in the floccular white matter. Retrograde tracing and analysis of neurobiotin-filled BIN neurons showed that each BIN neuron has widespread projections throughout the flocculus. In addition, using anterograde and retrograde tracing we identified afferents that make multiple VGluT2+ synaptic contacts on the soma and proximal dendrites of BIN neurons, and that arise bilaterally from neurons in the antero-medial medullary reticular formation. In summary, our data show that the BIN represents a unique novel inhibitory afferent system to the flocculus that complements inhibition by Golgi cells. We propose that the BIN cells play a unique role in the integration of vestibular and visuo-motor mossy fiber input, and may be a specific adaptation to appropriately modulate Purkinje cell activity during compensatory eye movements and compensatory eye movement adaptation.

Disclosures: D. Jaarsma: None. **F. Blot:** None. **M. Schonewille:** None. **Z. Gao:** None. **T.J.H. Ruigrok:** None. **C.I. De Zeeuw:** None.

Poster

719. Cerebellum: Anatomy, Cytoarchitecture, and Connectivity

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Support: CIHR MOP 299921

FRQNT NC-189153

Title: Characterization of Purkinje cell axonal torpedoes during postnatal development in mice

Authors: *D. LANG-OUELLETTE, L. LJUNGBERG, A. YANG, A. J. WATT;
Dept. of Biol., McGill Univ., Montreal, QC, Canada

Abstract: Information is carried from the cerebellar cortical microcircuit via action potentials propagated along Purkinje cell axons. In several human neurodegenerative diseases, as well as animal models, focal swellings in the axons of Purkinje cells − torpedoes − have been observed, often during disease progression when Purkinje cell loss occurs. How axonal torpedoes affect cerebellar function is poorly understood: both neurodegenerative and neuroprotective roles have been suggested. Interestingly, torpedo-like axonal swellings have also been transiently observed in the developing cerebellum. The role of developmental torpedoes in cerebellar information processing is even less understood than those in disease.

To elucidate the role of developmental Purkinje cell axonal torpedoes in the maturing cerebellum, we studied postnatal L7-*tau*-eGFP transgenic mice which label Purkinje cell axons. We found a transient increase in the number of torpedoes during the second postnatal week of development: peaking at P11 when up to ~40% of Purkinje cells had axonal torpedoes. Since Purkinje cell apoptosis occurs during development, we wondered whether torpedoes could be precursors of dying Purkinje cells. However, since we found that the majority of Purkinje cell death occurs prior to P9, when torpedo numbers were low, we conclude that developmental torpedoes are predominantly observed on the axons of Purkinje cells that survive into adulthood. Next, we wondered whether torpedoes were associated with axonal reorganisation, since developing axons undergo dramatic pruning during postnatal development. However, only a fraction of developmental torpedoes were found at branch points (~7% at P11). Furthermore, microglia were not enriched around torpedoes, as might be expected if they were associated with axonal pruning or cell death, and most torpedoes were myelinated. The accumulation of disorganized neurofilaments is a hallmark feature of disease-related axonal torpedoes. We observed enriched neurofilament in developmental torpedoes, which suggests that they may be structurally similar to torpedoes found on Purkinje cell axons in disease states.

Our findings show that Purkinje cell axonal torpedoes exist transiently in the developing mouse cerebellum, but are unlikely to be associated with cell death or axonal pruning. Furthermore, we find that developmental axonal torpedoes share common structural features with disease-related torpedoes, which may indicate that they serve a similar function in these different states of the cerebellar circuit.

Disclosures: D. Lang-Ouellette: None. **L. Ljungberg:** None. **A. Yang:** None. **A.J. Watt:** None.

Poster

719. Cerebellum: Anatomy, Cytoarchitecture, and Connectivity

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NIH P40 RR018604 (Center for Neuroanatomy with Neurotrophic Viruses)

Title: Nucleo-cortical neurons of the parvocellular interpositus target the Purkinje cell layer of the paraflocculus.

Authors: *G. J. WOJACZYNSKI[1], J. P. CARD[2];
[2]Neurosci., [1]Univ. of Pittsburgh, Pittsburgh, PA

Abstract: A central tenet of cerebellar processing has been the existence of a universal circuit motif that extends throughout the cerebellar cortex. However, differences in cytoarchitectural organization and afferent innervation observed across the deep cerebellar nuclei (DCN) imply that not all of the deep nuclei display identical synaptic relationships with the cortex, as has been previously thought. One aspect in which the DCN could differ is their recurrent projections to the cortex. For example, recent studies have shown that there exists a glycinergic nucleo-cortical projection stemming from the dentate nucleus which has not been observed thus far in other subdivisions of the DCN. We therefore tested the uniformity of nucleo-cortical projections by injecting an anterograde tracer, BDA, into a cytoarchitecturally and connectionally unique subnucleus of the DCN, the parvocellular interpositus nucleus (pcIP), and analyzing the terminal morphology and neurotransmitter content of nucleo-cortical projections in adult male rats. We demonstrate here the existence of a novel nucleo-cortical projection that originates from the pcIP and targets the paraflocculus. This projection arborizes immediately subjacent to the Purkinje cell (PC) layer, parallel to the orientation of PC dendrites, and does not match any previous descriptions of nucleo-cortical fibers. Though the majority of synaptic swellings were in the immediate vicinity of the PC layer, occasional swellings were also found within the deeper granular layer and the molecular layer. Immunocytochemical and ultrastructural analyses revealed that these fibers are excitatory, similar to recurrent nucleo-cortical projections from principal projection neurons in other subdivisions of the DCN. This projection was not seen when BDA injections were made outside of the pcIP, indicating that this projection is unique to the pcIP/paraflocculus. This unique connection suggests that the entirety of the cerebellar cortex may not function identically and that regions of the cortex and DCN are anatomically specialized to support different cerebellar functions.

Disclosures: G.J. Wojaczynski: None. **J.P. Card:** None.

Poster

719. Cerebellum: Anatomy, Cytoarchitecture, and Connectivity

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Title: Purkinje cell collaterals enable output signals from the cerebellar cortex to feed back to Purkinje cells and interneurons

Authors: *S. RUDOLPH[1], L. WITTER[1], R. T. PRESSLER[2], S. LAHLAF[1], W. G. REGEHR[1]; [1]Neurobio., Harvard Med. Sch., Boston, MA; [2]Dept. of Neurosciences, Case Western Reserve Univ. Sch. of Med., Cleveland, OH

Abstract: Purkinje cells (PCs) provide the sole output from the cerebellar cortex. Although PCs are well characterized on many levels, surprisingly little is known about their axon collaterals and their target neurons within the cerebellar cortex. It has been proposed that PC collaterals play an important role in early development, where they transiently control circuit assembly. However, after development functional PC to PC connections are thought to be pruned. Here, we find that all PCs have collaterals well into adulthood in mice. PC collaterals are restricted to the parasagittal plane, and most synapses are located in close proximity to PCs, although some are present also in the molecular layer. Using optogenetics and electrophysiology we find that in juveniles and adults PCs make synapses onto other PCs, as well as onto some other types of interneurons. These findings establish that PC output can feed back and regulate numerous circuit elements within the cerebellar cortex. Notably, collaterals are well suited to contribute to processing in parasagittal zones by suppressing firing of diverse neuron types within the cerebellar cortex.

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Poster

719. Cerebellum: Anatomy, Cytoarchitecture, and Connectivity

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Support: NIH R01-NS045193

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F31 NS089303-01

Title: High dimensional neuroanatomical and behavioral analysis for probing cerebellar involvement in nonmotor function

Authors: T. D. PEREIRA[1], J. W. METZGER[1,2], B. DEVERETT[1,2,3], T. J. PISANO[1,2,3], A. BADURA[1,4], *S. S.-H. WANG[1,2];
[1]Princeton Neurosci. Inst., [2]Mol. Biol., Princeton Univ., Princeton, NJ; [3]Rutgers-Robert Wood Johnson Med. School-Princeton Univ. MD/PhD Program, Rutgers Univ., New Brunswick, NJ; [4]Netherlands Inst. for Neurosci., Amsterdam Zuidoost, Netherlands

Abstract: Genetically encodable Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) allow reversible inactivation of identified cell types in freely-moving animals on a time scale of hours. To understand the role of specific cerebellar regions in guiding behavior during development and adulthood, we have developed detailed quantitative approaches for analyzing the extent of DREADD inactivation and the consequent pattern of behavioral perturbation.
In both juvenile and adult mice, we performed cerebellar lobule-specific injections of AAV8-hSyn-hM4D(Gi)-mCherry to co-express an inhibitory DREADD (hM4D) and a fluorescent reporter (mCherry). After administration of DREADD agonist clozapine-N-oxide (CNO), mice were tested sequentially in five behavioral paradigms commonly used to model autism in mice: an elevated-plus maze, reversal in a swimming Y-maze, self-grooming, three-chamber social preference, and a virtual reality-based working memory task. In each case, direct monitoring and video recording were used to acquire subsecond-resolution measurements of animal trajectory for offline analysis. To define different dimensions of autism-like phenotypes, we used principal components analysis to identify patterns of animal-to-animal covariation encompassing multiple behavioral measurements. In this way we created a basis set of "eigenbehaviors" constructed from performance in unperturbed mice, which we used to quantify lobule-specific inactivation.

These behaviors constitute a multidimensional autism-like phenotype.
We next sought to associate behavioral phenotypes with spatial patterns of DREADD expression. We used serial two photon (STP) reconstruction to render anatomical volumes complete with precisely mapped regions of expression. Our developed image processing pipeline builds aligned volumes from these STP stacks and registers the volumes to a standard mouse brain coordinate system (Allen CCFv2). Comparison of anatomical representations to behavioral phenotypes will test the hypothesis that lobule-specific perturbation of cerebellar regions leads to multi-dimensional effects on behavior. Our experimental and analytical framework enables quantitative linkage of neuroanatomy to behavior.

Disclosures: T.D. Pereira: None. **J.W. Metzger:** None. **B. Deverett:** None. **T.J. Pisano:** None. **A. Badura:** None. **S.S. Wang:** None.

Poster

719. Cerebellum: Anatomy, Cytoarchitecture, and Connectivity

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BRAIN U01 NS090541

Title: Postnatal disruption of cerebellar activity causes autism-like phenotypes in adulthood

Authors: *A. M. BADURA[1,2], J. W. METZGER[2,3], T. J. PISANO[2,3,6], B. DEVERETT[2,3,6], T. D. PEREIRA[2], S. KOAY[2], C. D. BRODY[2,4], D. W. TANK[2,5], S. S.-H. WANG[2,3];
[1]Netherlands Inst. For Neurosci., Amsterdam Zuidoost, Netherlands; [2]Princeton Neurosci. Inst., [3]Mol. Biol., [4]Howard Hughes Med. Inst., [5]Bezos Ctr. for Neural Dynamics, Princeton Univ., Princeton, NJ; [6]Rutgers-Robert Wood Johnson Med. School-Princeton Univ. MD/PhD Program, Rutgers Univ., New Brunswick, NJ

Abstract: Cerebellar lobules VI/VII and crus I/II form reciprocal loops with neocortical regions associated with executive functions (Wang et al, 2014). To test the functional significance of those connections we used DREADDs to disrupt neural activity of these cerebellar regions during adulthood or postnatal development, and measured the consequences in two major domains: (1) social choice and behavioral inhibition; and (2) cognitive flexibility.
We made lobule-specific injections of AAV8-hSyn-hM4D(Gi)-mCherry to achieve expression in molecular layer interneurons (MLIs), a major source of inhibition to Purkinje cells, which themselves send inhibitory projections to thalamus and neocortex in an organized map. For developmental inactivation, mice were injected with the virus at postnatal day 21 (P21), and received DREADD agonist clozapine-N-oxide (CNO) from P30 through P56. For acute inactivation, mice were injected at P42, followed by testing at P56 with CNO administered through i.p. on the day of testing. The inhibitory action of CNO on DREADD-expressing MLIs was confirmed in acute cerebellar brain slices, as well as through indirect effects on Purkinje cell firing in vivo in awake mice. To analyze behavioral data, we used principal component analysis (see abstract by Pereira, Metzger et al.).
In lobule VI, CNO administration in adult mice led to increased perseveration. The mice also showed reduced performance and bias in a virtual reality-based working memory task. Tasks were not affected by juvenile administration of CNO. These results suggest that lobule VI is an active part of brainwide circuitry for cognitive flexibility.
Developmental effects were seen from perturbations of crus I, crus II, and lobule VII. After unilateral AAV injections of crus I, CNO administration during development led to reduced social interaction in a three-chamber test and impaired reversal learning in a Y-maze, consistent with a role for crus I in social and cognitive maturation. Unilateral crus II perturbation led to reductions in movement in the elevated-plus maze and three-chamber task, as well as reduced social preference in the three-chamber test. None of these effects were seen with adult CNO treatment. In lobule VII, juvenile treatment with CNO decreased exploratory activity in the elevated-plus maze and increased social behavior in the three-chamber test. Opposite effects were seen in acute adult treatment with CNO, suggesting that lobule VII contributes to maturation and acute function of exploratory behavior.
These experiments provide direct evidence for the developmental diaschisis hypothesis of cerebellar contributions to autism-like phenotypes.

Disclosures: **A.M. Badura:** None. **J.W. Metzger:** None. **T.J. Pisano:** None. **B. Deverett:** None. **T.D. Pereira:** None. **S. Koay:** None. **C.D. Brody:** None. **D.W. Tank:** None. **S.S. Wang:** None.

Poster

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Nancy Lurie Marks Family Foundation

F31 NS089303

Title: Whole-brain mapping of cerebellar output pathways using transsynaptic viruses and light-sheet microscopy

Authors: *T. J. PISANO[1,2,3], S. DEIVASIGAMANI[1,2], J. C. LEE[1,2], B. DEVERETT[1,2,3], E. ENGEL[1], L. ENQUIST[2], S. S.-H. WANG[1,2];
[1]Princeton Neurosci. Inst., [2]Mol. Biol., Princeton Univ., Princeton, NJ; [3]Rutgers-Robert Wood Johnson Med. School-Princeton Univ. MD/PhD Program, Rutgers Univ., New Brunswick, NJ

Abstract: The cerebellum and neocortex exchange information in a region-specific manner via closed, bidirectional loops. In the ascending direction, cerebellar projections pass from Purkinje cells of the cerebellar cortex to the deep cerebellar nuclei (DCN) to thalamic and other midbrain areas before finally reaching the neocortex. To date, these pathways have been mapped largely using classical tracers that do not reveal polysynaptic connectivity. We are using a combination transsynaptic tracing viruses, iDISCO tissue clearing (Renier et al. 2014, Cell 159:896), and light-sheet fluorescence microscopy (LaVision Biotec., Bielefeld, Germany) to reconstruct and quantify cerebello-thalamo-neocortical projection patterns on a whole-brain scale without need for tissue sectioning.
We injected the anterogradely-transported herpes simplex virus 1 H129 CMV-EGFP (HSV1-H129, $2.7x10^4$ to $8.0x10^4$ PFUs, Wojaczynski et al. 2015 Brain Struct. Func. 220:3) into cerebellar lobule VI. Coinjection with Cholera Toxin B revealed that injection sites were 300 - 1200 micrometers wide. We detected viral antigen immunohistochemically (DAKO B0114) in the DCN at 37 hours post-injection, in the thalamus at 41 and 49 hours, and in the neocortex after 54 hours, consistent with the expected cerebello-thalamo-neocortical path. Corresponding with specific injection sites, we found expression in thalamic areas with nonmotor associations (lateral dorsal, medial dorsal, and parafascicular nuclei) and ventral tegmental area, nucleus accumbens, amygdala, and hypothalamus.
We also investigated the origins of pathways to specific neocortical targets by injecting a retrogradely-moving pseudorabies virus (PRV) strain of herpesvirus (PRV-Bartha CMV-EGFP, $2.4x10^4$ PFUs, Smith et al. 2000 PNAS 97:16) into specific neocortical regions. After 53 hours

we detected PRV antigen via immunofluorescence (Rb 134, Card et al. 1990, J. Neurosci. 10:1974) in Purkinje cells of multiple lobules, demonstrating the existence of spatially diverse cerebellar locations that converge upon a specific neocortical target. We are now using PRV to trace cerebellar pathways leading to mediodorsal nucleus of the thalamus, anterior cingulate cortex, and other forebrain regions thought to contribute to executive function.

Disclosures: T.J. Pisano: None. **S. Deivasigamani:** None. **J.C. Lee:** None. **B. Deverett:** None. **E. Engel:** None. **L. Enquist:** None. **S.S. Wang:** None.

Poster

719. Cerebellum: Anatomy, Cytoarchitecture, and Connectivity

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Nancy Lurie Marks Family Foundation

Title: Effects of disrupted cerebellar activity on dendritic development at distal neocortical sites.

Authors: *J. VERPEUT[1,2], A. TAO[1,2], C. HILDRETH[1], E. C. COPE[1,3], B. A. BRIONES[1,3], E. GOULD[1,3], S. S.-H. WANG[1,2];
[1]Princeton Neurosci. Inst., [2]Mol. Biol., [3]Psychology, Princeton Univ., Princeton, NJ

Abstract: Major sources of within-brain communication originate in the cerebellum, the most frequently observed site of structural abnormality in human autism-spectrum disorder. The cerebellum projects via thalamus in an organized manner to a wide variety of neocortical targets, including regions that contribute to cognitive and affective processing. In rodent frontal cortex, ascending axons from the thalamus synapse onto basal but not apical dendrites of pyramidal cells in layer 2/3 (Shigematsu et al., Cerebral Cortex 2015:1-16). To test whether cerebellar activity shapes neocortical dendritic arborization and spine formation through cellular plasticity mechanisms, we examined spine density in basal and apical dendrites in layer 2/3 pyramidal neurons of prelimbic (corresponding to medial prefrontal cortex) cortex in heterozygous L7-tuberous sclerosis 1 (L7-Tsc1) mice, which have alterations in Purkinje cell firing activity, abnormal social interactions, and repetitive behaviors (Tsai et al. 2012, Nature 488:647). We assessed neocortical dendritic and spine morphology using small-molecule lipophilic (DiI) labeling. Adult male L7-Tsc1 heterozygous mice had an increased density of total spines on basal dendrites compared to wild-type (WT) mice (20 neurons in 4 L7-Tsc1 mice, 25 neurons in

5 WT mice). However, the density of spines on apical dendrites was not different between heterozygotes and wild-type (20 neurons in 4 L7-Tsc1 mice, 25 neurons in 5 WT mice). These results are consistent with the hypothesis that cerebellar activity is necessary for normal pruning of spines at distal neocortical sites, potentially accounting for the ability of early-life cerebellar injury to cause autism-like pathologies. To test whether local perturbation can also influence dendritic structure in specific neocortical targets, we are now using adeno-associated viral vectors to express Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) to alter cerebellar activity in specific cerebellar lobules. These experiments will test the hypothesis that input from specific lobules of the cerebellum during sensitive periods of development can influence the growth and maturation of specific distal forebrain circuitry.

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Poster

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Topic: E.02. Cerebellum

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Title: Purkinje cell to granule cell synapse provide feedback to the input layer of the cerebellar cortex

Authors: ***C. GUO**, L. WITTER, S. RUDOLPH, H. ELLIOTT, K. ENNIS, W. REGEHR; Neurobio., Harvard Univ., Boston, MA

Abstract: The flow of information through the cerebellar cortex is primarily feed-forward: mossy fiber inputs activate granule cells, which in turn drive Purkinje cells, the sole output neurons of the cerebellar cortex. Inhibition onto granule cells is thought to play a key role in gating the flow of signals into the cerebellum, and it is thought that Golgi cells are the only interneurons that inhibit granule cells. Here we show that Purkinje cells synapse onto granule cells via axon collaterals, thus providing a route for direct output to input layer feedback in the cerebellar cortex. We propose that this non-canonical feedback could regulate excitability of the input layer, maintain sparse coding and mediate temporal integration.

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Poster

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Topic: E.02. Cerebellum

Title: A novel cerebellar molecular layer interneuron inhibited by Purkinje cell feedback

Authors: *J. KIM, G. J. AUGUSTINE;
LKC Med. School-NTU, Singapore, Singapore

Abstract: Although the organization of cerebellar cortical circuits is relatively well known in comparison to other brain areas, some potentially important synaptic connections remain unexplored. Here we have examined the function of a novel inhibitory synaptic circuit between Purkinje cells (PC) and molecular layer interneurons (MLIs). High-speed optogenetic circuit mapping (PNAS 104: 8143), using brain slices from transgenic mice selectively expressing channelrhodopsin-2 in PCs (Front. Neural Circuits 7:160), revealed the presence of a subset of MLIs (~16%) located in the inner third of the molecular layer that receive strong, fast and monosynaptic inhibitory inputs from PCs. These MLIs receiving PC feedback had several electrical characteristics – including larger hyperpolarization-activated current (Ih), larger membrane capacitance, and more hyperpolarized resting membrane potential – different from those of MLIs not receiving PC input. MLIs receiving PC input also had high input resistance, suggesting the absence of electrical coupling with neighboring MLIs. Consistent with this interpretation, the gap junction blocker mefloquine (Cell Rep. 7: 1601) selectively increased the input resistance of basket cells but not MLIs with PC input. Further, dye coupling measured with neurobiotin was present in basket cells but absent in MLIs receiving PC feedback. Thus, we conclude that MLIs with PC input do not participate in the gap junction network of MLIs. Further, the morphology of MLIs with PC input is clearly different from other MLIs: although they were located in the inner third of the molecular layer, they formed neither baskets around PCs nor had horizontal processes characteristic of basket cells. These results indicate that MLIs that receive PC feedback inhibition represent a novel type of MLI. The unique circuit formed between PCs and these MLIs may play an important role in cerebellar information processing by synchronizing neighboring PCs via disinhibition.

Disclosures: J. Kim: None. **G.J. Augustine:** None.

Poster

719. Cerebellum: Anatomy, Cytoarchitecture, and Connectivity

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 719.15/RR16

Topic: E.02. Cerebellum

Support: CNRS

Title: Calcium imaging reveals coordinated simple spike pauses in populations of Cerebellar Purkinje cells

Authors: J. E. RAMIREZ[1], *B. M. STELL[1,2];
[1]Univ. Paris Descartes, Paris, France; [2]CNRS, Paris, France

Abstract: The cerebellum is thought to coordinate movement by processing sensorimotor information in the cerebellar cortex before relaying its output to other brain structures. Since all information processed by the cerebellar cortex converges on Purkinje cells (PCs), the ability to record spiking from identified populations of these cells presents new possibilities for understanding cerebellar processing. Here we show that somatic calcium imaging can be used in slices and in vivo to easily distinguish calcium fluctuations generated by complex spikes from those generated by trains of simple-spikes (SS). Moreover, the estimated calcium influx from a single SS is shown to be remarkably consistent between PCs ($\Delta F/F_0$ of 0.1 +/- 0.03%, n = 6) and therefore permits monitoring the relative changes in SS firing frequency. We report that transitions into and out of pauses in SS activity (lasting longer than three median inter-spike intervals) are correlated between PCs in the sagittal plane. The source of this organization is shown to be presynaptic GABAergic network and blocking $GABA_A$Rs with gabazine abolishes the synchrony (permutation tests, $p < 0.05$ in control; $p > 0.05$ in treatment; n = 7 pairs). Also, paired cell-attached recordings of adjacent PCs in sagittal slices (n = 72 pairs) showed that this synchrony decays abruptly after a distance of ~200 um between PCs, consistent with the mean length of GABAergic interneuron axons in the cerebellar molecular layer (de San Martin et al. 2015). Thus, this method reveals that simple-spike pauses are organized in groups of neighboring PCs by presynaptic interneurons to maximize the effect of PC inhibitory synapses on their common downstream targets.

Disclosures: J.E. Ramirez: None. **B.M. Stell:** None.

Poster

719. Cerebellum: Anatomy, Cytoarchitecture, and Connectivity

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Topic: E.02. Cerebellum

Support: ERC Advanced Grand SINGLESITE

ENP Post Doc Grant

Title: Exploring molecular layer interneuron network activity in the cerebellar cortex *In vivo* using the calcium indicator protein GCaMP6f

Authors: *M. GRAUPNER, A. JALIL, I. LLANO;
Univ. Paris Descartes, Brain Physiol. Lab, CNRS, Paris Cedex 06, France

Abstract: Networks of inhibitory neurons coupled through chemical and/or electrical synapses are abundant in the brain and have been shown to exhibit synchronous, oscillatory activity. Such oscillations have been proposed to provide a reference signal for temporal encoding, sensory binding of features, and storage and recall of information. Molecular layer interneurons (MLIs) of the cerebellum are interconnected by GABAergic chemical synapses and by electrical synapses. These connections have been shown to promote synchrony and shape the temporal precision of action potentials. However, the activity patterns and the role of the MLI network in the intact animal remain unknown. While fluorescent calcium sensors such as GCamMP6f provide a means to track the activity of neuronal populations *in vivo*, the interpretation of such signals from tonically firing inhibitory neurons with potent calcium-binding proteins, such as parvalbumin remains challenging.

In order to shed light on the activity patterns occurring in and the functional role of the interneuron network *in vivo*, we perform calcium imaging experiments from ensembles of MLIs in lobule V of the cerebellar vermis of ketamine/xylazine-anethetized mice (P30-50). The *PV-Cre* driver line was crossed with the *Ai93 GCaMp6F-TIGRE* promoter line which led to ubiquitous GCaMP6f expression in MLIs accompanied by sparse expression in granule and Purkinje cells. To study the link between the calcium signal and spiking activity in cerebellar MLIs we conducted simultaneous cell-attached recordings from single interneurons using patch-clamp pipettes. Sensorimotor-driven synaptic inputs to MLIs were simulated using parallel fiber stimulation with theta-glass pipettes inserted in the molecular layer.

We characterize the information carried by the calcium signal about the underlying spiking activity for the tonically firing MLIs in the anesthetized animal. Analysis of spontaneous activity epochs and activity evoked by parallel fiber stimulation allows us to extract the minimum change in firing that can be faithfully detected by GCaMP6f. Our measurements allow us to discern the calcium signal component generated by MLI spikes from the part of the signal due to synaptic

inputs. We furthermore characterize the magnitude and spatial extend of correlated activity changes across neighboring MLIs conveyed by the fluorescent signal. Our results provide a key step to measure and interpret activity patterns and synchronization regimes of the MLI network. Deciphering MLI activity is required to advance our understanding of this interconnected microcircuit and its involvement in generating coordinated movement in mammals.

Disclosures: **M. Graupner:** None. **A. Jalil:** None. **I. Llano:** None.

Poster

719. Cerebellum: Anatomy, Cytoarchitecture, and Connectivity

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Topic: E.02. Cerebellum

Support: This work is supported by a GRF grant from the Research Grant Council of the Hong Kong Special Administrative Region Government (CityU 11100015)

Title: Alternation of transcription factor gene expression in cerebellar Purkinje cells disrupts the synchrony of simple and complex spike in deep cerebellar nuclei

Authors: ***G. KUMAR**[1], W. Y. TAM[1], K. M. KWAN[2,3], C. H. E. MA[1,4];
[1]City Univ. of Hong Kong, Kowloon, Hong Kong; [2]Sch. of Life Sciences,The Chinese Univ. of Hong Kong, Shatin, Hong Kong; [3]State Key Lab. of Agrobiotechnology (CUHK), The Chinese University of Hong Kong, Hong Kong; [4]Ctr. for Biosystems, Neuroscience, and Nanotechnology, City Univ. of Hong Kong, Kowloon, Hong Kong

Abstract: The cerebellar Purkinje cell is inimitable and complex neuron acts as a computation center in the brain. It receives convergent projections from all other cortical neurons and providing the sole output signal to deep cerebellar nuclei (DCN). DCN acts as relay center to receive all the information from Purkinje cells (PCs) and convey to premotor nuclei. These DCN neurons integrate synaptic inputs with their own spontaneous activity to generate motor signals for locomotion and fine-tuning of movement. The impairment of Purkinje cells may modify excitatory synaptic transmission electrophysiology i.e. spontaneous simple and complex spike firing rate, duration of spike, and the duration of the simple spike pause after the complex spike. Intrinsic dynamics of neurons is a critical step in the process of network behavior, synaptic integration, and information processing in the brain. Previous studies have reported that partial loss of PC was associated with ataxia-like phenotype in mice due to reduced Purkinje neuron activity and increased DCN intrinsic excitability. Hence, electrophysiological study of DCN will give insight into dissecting the motor neurocircuitry pathway of output signal from the central

nervous system to the peripheral nervous system. We therefore hypothesized that ablation of a transcription factor in PC cells affects the DCN neurons spike firing and modulating the motor output signal. In the present study, conditional mutant mice with ablation of a transcription factor in cerebellar PCs exhibited lack of motor coordination assessed by rotarod. Neurocircuitry pathway of motor output was further confirmed by recordings the DCN neurons activity in mutant mice. The results of present study showed that mutant mice exhibited significant decrease in retention time on rotarod as compared with control ($p<0.05$). Recording of DCN neurons exhibited significant changes in simple and complex spike frequency, since a subset of neurons displayed bursts of action potentials in the mutant mice which required further confirmation. These ongoing studies suggest that ablation of transcription factor alter motor signal by modulating the DCN intrinsic excitability. Further studies will focus on elucidating the cerebellum neurocircuitry in mutant mice.

Disclosures: **G. Kumar:** None. **W.Y. Tam:** None. **K.M. Kwan:** None. **C.H.E. Ma:** None.

Poster

719. Cerebellum: Anatomy, Cytoarchitecture, and Connectivity

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Topic: E.02. Cerebellum

Support: Wellcome Trust WT094077MA

ERC AdG 250345

Title: Principles of excitatory and inhibitory connectivity in the cerebellar molecular layer *In vivo*

Authors: ***C. ARLT**[1], C. D. WILMS[2], M. HAUSSER[1];
[1]Wolfson Inst. For Biomed. Res., Univ. Col. London, London, United Kingdom; [2]Scientifica, Uckfield, United Kingdom

Abstract: Molecular layer interneurons (INs) and Purkinje cells (PCs) in the cerebellar cortex receive common excitatory granule cell input, resulting in feed-forward inhibition (FFI) of PCs by INs. Climbing fibers (CFs) are thought to instruct cerebellar learning at the parallel fiber synapse onto PCs, but also influence INs: *in vitro* experiments have shown that CF input excites INs via glutamate spillover, but can also cause IN inhibition, by recruiting GABAergic interactions among INs. However, the spatiotemporal patterns of activity emerging from CF, IN and PC interactions remain poorly understood. Using simultaneous patch-clamp recordings from

INs and PCs guided by two-photon microscopy in anesthetised mice, we studied higher-order functional connectivity of CFs, INs, and PCs using cross-correlation analysis of PC complex spikes, simple spikes, and IN spikes.
We find that single spontaneous IN spikes can correlate with PC spike rate decreases (PC rate z-score of <-3 0-10 ms after IN spike, n = 24 of 56 pairs), and that induced spikes in a single IN can cause PC inhibition to a similar degree. IN-PC functional connectivity depends on vertical distance: INs with inhibitory effects on PCs are closer to the PC layer than INs without an effect (mean distance from PC layer: 106 ± 43 versus 135 ± 30 μm, p = 0.006). In a subset of functionally connected IN-PC pairs (n = 6), PC inhibition after the IN spike is preceded by a narrow increase in spike rate (PC rate z-score of >3 -10-0ms from IN spike), indicative of the IN and PC receiving common granule cell input and the IN delivering FFI to the PC.
CF input assessed by measuring complex spikes (CSs) in PCs results in a variety of IN rate changes, with INs either exhibiting rate increases (IN rate z-score of >3 0-20ms after CS, n = 13), delayed decreases (z-score of <-3 10-30ms after CS, n = 8), or increases followed by decreases (n = 3). IN responses to CF input also depend on vertical distance: Excited INs are closer to the pia, whereas inhibited INs are closer to the PC layer (mean IN distance to PC layer: 126 ± 39 versus 78 ± 33 μm, p = 0.03). This organisation is in line with a preference for INs higher up to inhibit INs lower in the molecular layer, which we have assessed directly by dual recordings from IN-IN pairs.
Finally, we analyse the overlap of functional CF-IN and IN-PC connectivity, and find that there is no preference for connected IN-PC pairs to receive common excitatory CF input, but rather for INs inhibiting a PC to be inhibited upon CF input. This motif is especially prevalent in IN-PC pairs with common granule cell input, which may help to maintain FFI during plasticity, controlling the ratio of weights of granule cell inputs to INs and PCs.

Disclosures: **C. Arlt:** None. **C.D. Wilms:** None. **M. Hausser:** None.

Poster

719. Cerebellum: Anatomy, Cytoarchitecture, and Connectivity

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Topic: E.02. Cerebellum

Support: Human Brain Project HBP 604102

Title: Large-scale realistic modeling reveals spatiotemporal dynamics of cerebellar granular layer responses to mossy fiber bursts

Authors: *S. CASALI[1], M. TOGNOLINA[1], E. D'ANGELO[1,2];
[1]Brain and Behavioral Sci., Univ. of Pavia, Pavia, Italy; [2]Brain Connectivity Center, C. Mondino Natl. Neurolog. Inst., Pavia, Italy

Abstract: The investigation of the internal algorithm of the cerebellar microcircuit is proving extremely challenging from an experimental perspective largely due to the difficulty of recording from multiple individual neurons simultaneously. Detailed computational modelling can provide insight into the structure of network computation. The NEURON-Python platform has been used to develop a large scale realistic model of cerebellar granular layer (GL), including connectivity and representations of its main neuronal types, Granule cells (GrCs) and Golgi cells (GoCs), and of their synapses (D'Angelo *et al.*, 2001; Nieus *et al.*, 2006, 2014; Diwakar *et al.*, 2009, 2011; Solinas *et al.*, 2007*a*,*b*, 2010). The model has been validated against observations at the mesoscopic scale (including VSD/MEA recordings and two-photon Ca^{2+} imaging *in vitro* and LFP recordings *in vivo*). The main goal of the present work is to demonstrate that spatiotemporal dynamics taking place in the GL at a mesoscopic scale can be understood as emergent phenomena, caused by the complex interaction occuring among microscopic variables including the topology of intercellular connectivity and the neurophysiological properties of neurons and synapses. Our results show that (1) the center-sorround profile describing the ratio between excitation and inhibition observed in cerebellar slices depends on the spatial arrangement of GrC - GoC connectivity; (2) the interaction between different spots of activation generates antithetical response patterns, combined excitation and inhibition (logical operators AND and XOR); (3) transverse and parasagittal connectivity can be differentially modulated by the GrC-GoC ascending axon (AA) and parallel fibers (PF), by GoC-GoC gap-junctions, and by GoC-GoC inhibitory interneurons. Interestingly, the AA confines feedback inhibition to local GL clusters, while PFs have weak effect on GoC activity. Conversely, relevant local effects are generated by GoC-GoC connections. (4) The GL as a whole can generate coherent oscillations in response to random background when two conditions are met: the MF input conveyed to GoCs is weak and GoCs do not inhibit each other; (5) different frequency patterns of random MF background can tune the response properties of the GL toward a wide range of inputs, making the cerebellar input-stage adaptive to various forms of incoming informations. In conclusion, our results show that reconstructing the biophysical and structural network features can predict the emergence of the spatiotemporal properties that let the GL to be defined as a reconfigurable dynamical system.

Disclosures: S. Casali: None. **M. Tognolina:** None. **E. D'Angelo:** None.

Poster

720. Physiology of Basal Ganglia Systems

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Program#/Poster#: 720.01/RR21

Topic: E.03. Basal Ganglia

Support: DA006886

Title: Processing of individual body parts by direct (drd1) and indirect (drd2) pathway neurons on the dorsal striatum

Authors: *K. COFFEY[1], M. WEST[2];
[1]Rutgers Univ., New York, NY; [2]Rutgers Univ., Piscataway, NJ

Abstract: The dorsolateral or sensorimotor striatum (DLS) is a prominent target of research on control of voluntary movement, sensorimotor integration. The DLS is comprised mainly (95%) of type IIb, medium spiny projection neurons. These neurons receive direct monosynaptic projections from primary somatosensory (S1) and motor (M1) cortices in a similar manner across species. Clusters of medium spiny neurons (MSNs) show firing rate (FR) increases related to sensorimotor activity of individual body parts in monkeys and rats. To this date, this work has yet to have been extended to mice. These striatal MSNs are thought to output through the "direct and indirect pathways". According to this model, "direct pathway" MSNs project gabba to the substantia nigra pars reticulata (SNr) and the globus pallidus internal segment (GPi). The inhibition of GPi and SNr leads to disinhibition of the thalamic glutamatergic neurons, which project to the cortex and is thought to facilitate movement. Conversely the "indirect pathway" MSNs inhibits the GABAergic neurons of the GPe, leading to a disinhibition of the glutamatergic neurons of the STN. This is hypothesized to result in the reduction of locomotor activity and movement. In addition to their distinct projections, MSNs of the direct and indirect pathway are characterized by the differential expression of dopamine (DA) receptors. D1 dopamine receptors (Drd1) are hypothesized to be expressed by direct pathway MSNs, whereas D2 dopamine receptors (Drd2) are expressed by indirect pathway MSNs. No one has yet attempted to reconcile the very well classified single body part (SBP) neurons with the primary functional tenet of the direct/indirect pathway hypothesis: that Drd1 neurons are active during movement, while Drd2 neurons are active during non-movement. Based on the theory alone, one might predict that single body part neurons are all Drd1 expressing MSNs witch reside in the direct pathway. However, the recent evidence showing Drd2 MSN activation during movement suggests that it is possible that both Drd1 and Drd2 MSNs could be single body part neurons. The present study first seeks to confirm that dorsolateral striatum in the mouse brain has an analogous sensorimotor function to that of primates and rats. We then seek to confirm that Drd1 MSNs primarily project to GPi, while Drd2 MSNs primarily project to GPe, and we also seek to determine for the first time if body part sensitivity of striatal MSNs can be discriminated unambiguously in the mouse. Finally, we seek to determine for the first time if these single body part neurons express Drd1 or Drd2 and reside in the direct and/or indirect pathways.

Disclosures: **K. Coffey:** None. **M. West:** None.

Poster

720. Physiology of Basal Ganglia Systems

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Topic: E.03. Basal Ganglia

Support: NIH GrantR01 NS083815

Title: Functionally dissecting the cortical control of direct versus indirect pathways in the striatum

Authors: *J. R. KLUG[1,2], H. A. HOFFMAN[1,2], F. OSAKADA[4], E. M. CALLAWAY[1,3], X. JIN[1,2];
[2]Mol. Neurobio. Lab., [3]Systems Neurobio. Lab., [1]Salk Inst. for Biol. Studies, La Jolla, CA; [4]Nagoya Univ., Nagoya, Japan

Abstract: The dorsal striatum and its excitatory inputs are involved in decision-making, especially action selection, initiation and learning. These abilities are compromised in human patients with neurological disorders, such as Parkinson's and Huntington's disease, affecting the striatum and its afferents. Spiny projection neurons (SPNs), the principal GABAergic projection neurons of the striatum, receive dense glutamatergic inputs from various cortical regions as well as thalamic nuclei. There are two intermixed subpopulations of SPNs: dopamine receptor 1 - containing (D1-SPNs, direct pathway) and dopamine receptor 2 - containing (D2-SPNs, indirect pathway). While the two major neural pathways constitute the core components of the classic model of the basal ganglia, very little is known about the connectivity and specificity of their excitatory inputs and role in behavior. In order to address these fundamental questions, we have used AAV-Cre-dependent helper viruses along with an EnvA-pseudotyped, glycoprotein (RG) - deleted recombinant rabies virus (SAD delta G-Rabies) expressing channelrhodopsin-2 (ChR2) to selectively activate monosynaptic inputs to D1- or D2-SPNs. The input pathway specific functional expression of ChR2 allowed for the determination of shared, overlapping excitatory populations to direct or indirect neurons in whole-cell brain slice electrophysiological recordings. Optic fiber based optogenetic stimulation in the freely moving mouse was utilized to examine the contribution of different cortical and thalamic regions to locomotor behavior, intracranial self-stimulation and operant sequence execution. These results provide new insight to how excitatory inputs connect to D1- and D2-SPNs, and determine the contribution of region- and pathway-specific corticostriatal subcircuits to behavior.

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Poster

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Topic: E.03. Basal Ganglia

Support: NIH Grant NS083815

Pioneer Postdoctoral Endowment Fund

Title: Feed-forward inhibition of nigrostriatal dopamine release by goal-directed action

Authors: *N. G. HOLLON, J. R. KLUG, H. LI, C. D. HOWARD, X. JIN;
Mol. Neurobio. Lab. J, Salk Inst. for Biol. Studies, La Jolla, CA

Abstract: Nigrostriatal dopamine is critically involved in voluntary movement, motivation, and reinforcement learning, but its precise role in instrumental action remains poorly understood. In the current work, we examined how self-initiated goal-directed action influences nigrostriatal dopamine transmission in the absence of any overt changes in an animal's external environment. Mice expressing channelrhodopsin in their dopamine neurons learned to press a lever on a continuous reinforcement schedule to deliver blue-light stimulation into their substantia nigra pars compacta. Performance of this free-operant optogenetic intracranial self-stimulation (opto-ICSS) was sensitive to changes in the action-outcome contingency, indicating that animals' behavior was goal-directed. Using fast-scan cyclic voltammetry, we monitored changes in dorsal striatal dopamine concentration during both opto-ICSS performance and subsequent playback of stimulations delivered in the same temporal sequence but not contingent upon any action. Stimulation-evoked dopamine release during opto-ICSS performance was markedly lower than that during non-contingent playback, indicating that self-initiated action causes a robust suppression of striatal dopamine release, even when driven by direct optogenetic stimulation of dopamine neurons. Omission probe trials revealed transient dips below baseline dopamine levels after non-reinforced lever presses, consistent with the idea of a feedforward inhibition triggered by the goal-directed action, likely through an efference copy. Probe trials with delayed stimulation or varied magnitude revealed that the action-induced suppression is precisely timed and well balanced to counteract the expected consequence of that action. Collectively, these findings demonstrate that nigrostriatal dopamine could signal prediction errors in action-outcome associations, and have fundamental implications in instrumental behavior and movement control.

Disclosures: **N.G. Hollon:** None. **J.R. Klug:** None. **H. Li:** None. **C.D. Howard:** None. **X. Jin:** None.

Poster

720. Physiology of Basal Ganglia Systems

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Program#/Poster#: 720.04/SS2

Topic: E.03. Basal Ganglia

Support: Swiss National Science Foundation

Title: Basal ganglia circuits for reinforcement and learning

Authors: *A. LALIVE, A. KREITZER;
Neurolog. Disorders, Gladstone Inst., San Francisco, CA

Abstract: Dopamine D1 receptor-expressing medium spiny neurons (D1-MSNs) in the dorsal striatum project to and inhibit the Substantia Nigra reticulata (SNr), forming the direct pathway of the Basal Ganglia (BG). Activation of this projection is sufficient to increase movement and drive locomotion. Interestingly, direct pathway activation can also drive real-time place preference, and mice readily learn to self-stimulate D1-MSNs, demonstrating their role in reinforcement. However, little is known about the locus or underlying mechanisms of reinforcement, which could include plasticity in the striatum or downstream targets. Here we designed a five-day training paradigm where mice could self-stimulate D1-MSNs. To determine if reinforcement occurred in striatum or downstream, we tested whether mimicking direct pathway stimulation effects on SNr could also drive reinforcement. Because inhibition of the SNr leads to disinhibition of target regions, including Substantia Nigra compacta (SNc), dorsal raphe, thalamus, and brainstem, we asked whether direct activation of SNr-recipient nuclei would be sufficient to drive reinforcement. Our results have delineated several potential targets of the direct pathway that are sufficient to drive reinforcement. We are now in the process of determining which of these circuits are necessary for direct pathway-mediated reinforcement, and finally testing the role of these circuits in learning to natural reinforcers.

Disclosures: **A. Lalive:** None. **A. Kreitzer:** None.

Poster

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Topic: E.03. Basal Ganglia

Support: NSF IOS1557987

Whitehall Foundation 2014-05-77

Title: Optogenetic stimulation of the dorsolateral striatum increases performance automaticity in cue-driven and response-driven tasks

Authors: *F. ŠTOCEK, A. C. G. CREGO, A. G. MARCHUK, K. S. SMITH;
Psychology and Brain Sci., Dartmouth Col., Hanover, NH

Abstract: Within the basal ganglia, the dorsolateral striatum (DLS) is known to play an important role in action selection and behavioral automatization. For example, in maze tasks, the DLS is involved in shaping "response" strategies in which choices are dictated by learned action routes. Response strategies can be achieved in principle by action selection at the outset of performance. Consistent with this notion, recording studies have identified a phasic burst of DLS activity at the onset of action sequences, which correlates with how fluid and fast the action sequence will be. To study the causality of this signal, rats were trained to execute a response strategy (run, turn, stop) for a sucrose reward on a plus-maze task. Optogenetic stimulations (channelrhodopsin) or inhibitions (halorhopsin) were applied at the start of runs and compared to manipulations during the middle of the run or during the full run. DLS stimulation at the start and for the full duration led to increased run speed and reduced deliberative head movements at the choice point. DLS inhibitions produced the opposite effects. This suggested that DLS activity at the start of a behavior carries influence over how automatic the behavior is. It remained unclear if this DLS activity promoted the selection of a learned action sequence, as though it were "chosen" from the start, or if it promoted the most optimal action routine on the task, in this case to execute a certain run-turn routine. To address this, we have begun to similarly evaluate the DLS on a task requiring animals to navigate towards visual cues that could only be identified at the choice point in the maze, after the run had been initiated. Preliminary, we find that DLS stimulation at the start of such runs increases the efficiency of runs on this task as well. In addition, DLS stimulation in the middle of the run, when the navigation choice must be made, also increased performance efficiency. The same was not true in the "response" task, in which optimal actions could be selected from the start of the run; mid-run stimulation in that condition had no effect on behavioral measures. Across both tasks, run accuracy was not altered. These findings highlight a preferential role for on-line DLS activity in shaping how optimal and/or fluid

a reinforced behavior is, whether that behavior involves active cognitive processing (i.e., locating and navigating towards a cue) or whether it might not (i.e., executing a response routine).

Disclosures: F. Štocek: None. **A.C.G. Crego:** None. **A.G. Marchuk:** None. **K.S. Smith:** None.

Poster

720. Physiology of Basal Ganglia Systems

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Topic: E.03. Basal Ganglia

Support: NSF IOS1557987

Whitehall Foundation 2014-05-77

Title: Exploring a causal role for the dorsolateral striatum in action-reward contingency shifts

Authors: *A. C. CREGO, K. S. SMITH;
Dartmouth Col., Hanover, NH

Abstract: In the basal ganglia, phasic bursts of activity at the onset of action sequences arise as those sequences are learned and become automatic. This pattern is particularly prevalent in the dorsolateral striatum (DLS) and is linked to the fluidity with which actions are performed. The relationship between increased DLS activity and fluid action performance can occur early in learning, suggesting a role for the DLS in the formation and maintenance of automatic actions. DLS loss-of-function studies show that it is necessary for the expression of automatic action sequences and habits once they have been acquired. However, it is unknown if on-line DLS activity is sufficient to strengthen action sequences; nor is it known, when new action patterns must be learned in place of old ones, whether engagement of DLS activity strengthens the previously learned (cached) behavior or instead promotes the formation of the newer, more optimal behavior? To address this issue, we leveraged the spatiotemporal precision of optogenetics to manipulate DLS activity at precise time points during an operant task, in which rats were exposed to a series of contingency shifts. Rats first learned an FR1 contingency, which was then suddenly shifted to an FR3 (upshift). After training on the FR3 schedule, rats were then suddenly returned to an FR1 (downshift). DLS was optogenetically stimulated using channelrhodopsin at the first lever press for both the (1) surprise upshift and (2) surprise downshift. Compared to controls, during the upshift, DLS stimulation resulted in fewer 1-press bouts versus 3-press bouts. Similarly, DLS stimulation during the downshift led to an increase in

1-press bouts, with fewer 3-press bouts. Though preliminary, this finding suggests that DLS stimulation at the start of an action tends to result in behavior that favors the more optimal action strategy for reward, in this case either a 1-press or a 3-press bout, rather than simply strengthening the most recently learned lever-press pattern or promoting perseverative pressing in general. In other words, DLS stimulation appeared to aid on-line behavioral flexibility and performance optimization. Finally, persistence in lever pressing was evaluated through a shift to a VI30 contingency protocol, in which reward was delivered independent of pressing. DLS stimulation resulted in animals showing less pressing than controls. This finding again suggests that DLS stimulation aided animals' sensitivity to the new contingency, resulting in a more optimal response strategy. Additional performance measures are being assessed.

Disclosures: A.C. Crego: None. **K.S. Smith:** None.

Poster

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Brain Research Foundation

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NIH T32 NS007222

Title: Precisely timed optogenetic inhibition of dopaminergic neurons during fine motor skill acquisition in rats

Authors: D. J. ELLENS[1], M. GAIDICA[2], A. R. HURST[1], *D. K. LEVENTHAL[1,4,3]; [1]Neurol., Univ. of Michigan, Ann Arbor, Ann Arbor, MI; [2]Neurosci. Grad. Program, [3]Biomed. Engin., Univ. of Michigan, Ann Arbor, MI; [4]Neurol., VA Ann Arbor Hlth. Syst., Ann Arbor, MI

Abstract: Striatal dopamine loss is the most prominent neurochemical finding in Parkinson Disease (PD), and dopamine replacement dramatically improves motor function in PD patients. Standard models of the basal ganglia, as well as the short duration response to levodopa, suggest that striatal dopamine acutely influences motor function. It is also clear, however, that striatal dopamine influences implicit learning processes. How these dual functions of dopamine are related to clinical parkinsonism is unclear, and difficult to tease out with interventions that

operate on timescales of hours (e.g., pharmacology) to days or years (e.g., lesions). Precisely timed optogenetic inhibition of midbrain dopaminergic neurons during acquisition and performance of a fine motor skill allows us to separate potential “learning” and “performance” roles of striatal dopamine. We employed a rat skilled reaching task with real-time computer vision to detect reach onset and initiate optogenetic suppression of dopamine neurons. Our preliminary results suggest that dopamine neuron suppression at reach onset does not influence reach trajectory, but reduces the likelihood that rats will perform future reaches. Our ongoing studies aim to distinguish motor from motivational effects of dopamine neuron suppression, define functional subpopulations of midbrain dopamine neurons with respect to skilled reaching, and examine how the timing of dopaminergic neuron activity influences motor skills.

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Poster

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Title: Distributed by asymmetric encoding of time in cortical and striatal networks

Authors: ***K. BAKHURIN**, V. GOUDAR, J. L. SHOBE, L. D. CLAAR, K. LEE, D. V. BUONOMANO, S. C. MASMANIDIS;
Neurobio., Univ. of California Los Angeles, Los Angeles, CA

Abstract: Telling time is fundamental to many forms of learning and behavior, including the anticipation of rewarding events. While the neural mechanisms underlying timing remain unknown, theoretical work has led to the hypothesis that the representation of time is an emergent property of the dynamic activity of neural networks. Computational models have largely focused on the representation of time in simulated networks that mimic cortical microcircuitry. However, motor timing deficits in Parkinson’s disease, experimental evidence from animals, and imaging studies in humans have pointed to a role for the striatum in temporal processing. Recent electrophysiological studies have demonstrated that the striatum may also encode time through moment-to-moment changes in spiking activity across its neural networks.

Despite these gains, a comparison of the extent to which cortical and striatal networks can represent time has not yet been performed. In this study, we made extracellular recordings of large neuron populations (55-120 simultaneously recorded units) in the striatum or prefrontal cortex (PFC) of awake, behaving mice that were trained to learn a fixed temporal relationship between odor cue presentations and the delivery of a reward. We used a machine-learning algorithm to quantify how well networks in each of these brain areas encoded the passage of absolute time along the cue-reward interval. While both cortical and striatal datasets were capable of encoding time, the striatum outperformed the PFC. In addition to decoding the elapsed time during the interval, we were able to use the population activity to predict, up to a second in advance, movement onset in the task. Again, this function was more robust in the striatum. Lastly, prediction of absolute time and movement onset time was dependent on the number of single units included in the analysis. These findings were recapitulated by repeating the same analyses in networks recorded simultaneously in both the PFC and striatum. This study reveals that time may be represented in a distributed manner, both within networks and across different regions of the brain. It also highlights the privileged role of the striatum in telling time, which is perhaps a result of its unique role as an integrator of activity from a diverse set of inputs.

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Poster

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Title: Behavioral changes and voltage imaging of cortical activity in a forced choice licking task using optogenetic inhibition of basal ganglia output in mice

Authors: *A. MORRISSETTE[1], P.-H. CHEN[1], P. Y. BORDEN[2], G. B. STANLEY[2], D. JAEGER[1];
[1]Biol., Emory Univ., Atlanta, GA; [2]Biomed. Engin., Georgia Tech., Atlanta, GA

Abstract: The modulation of cortical activity by basal ganglia output in behaving animals remains poorly understood. The substantia nigra pars reticulata (SNr) is the major source of inhibitory basal ganglia (BG) output in the mouse. It receives input from the direct and indirect basal ganglia pathways and transmits this activity via ascending projections (to the thalamus) and descending projections (to the superior colliculus and brainstem). Previous studies inactivating or ablating basal ganglia output in primates showed slowing of movement and little to no change in reaction time, however, these studies lack temporal specificity during animal behavior. In order to examine the effects of transient changes in basal ganglia output on cortical activity we expressed archaerhodopsin (eARCH3.0) or a novel inhibitory chloride channel (iC++) in the SNr for output inhibition with light stimulation. Behavioral impact of stimulation was assessed during open field spontaneous behavior and in a forced choice licking task. Further, we imaged cortical activity using the genetically encoded voltage indicator (GEVI) VSFP-Butterfly (VSFPB 1.2). Preliminary data suggest that unilateral optogenetic stimulation starting before or at the onset of go sensory cue in a left/right licking task leads to decreased reaction times and an increased number of licks to both left and right lick ports during the behavioral task. In open field behavior, contralateral rotations significantly increased during extended periods (5 min) of unilateral optogenetic inhibition, though mean velocity remained unchanged. Voltage imaging showed reliable cortical activation during licking behavior in motor cortex. Transient cortical activation was also seen following silencing of basal ganglia output, even in the absence of motor behavior. We will further examine cortical activity and behavior changes due to SNr stimulation during specific segments of the left/right licking task to determine effects of SNr silencing on movement initiation and suppression.

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Support: Magnus Bergvall

Kock

Crafoord

Olle Engkvist

Michael J Fox

Parkinsson Research Foundation

Parkinsson Foundation

Title: The role of corticostriatal circuits in transition of behavior

Authors: *J. SJÖBOM, M. TAMTÈ, P. HALJE, I. BRYS, P. PETERSSON;
Integrative Neurophysiol. and Neurotechnology, Lund Univ., Lund, Sweden

Abstract: In natural behavior, we smoothly change from one type of motor activity to another in a sequence of motor patterns that can be flexibly combined depending on behavioral context or the specific functional goal of the motor act. How such action sequences are constructed by neuronal circuits is largely unknown but corticostriatal circuits have long been thought to have a particularly important role in this type of organization of motor behavior.
To clarify the role of corticostriatal circuits in behavioral transitions we have investigated neuronal activity patterns in primary and secondary motor cortical areas, as well as in the lateral and medial volumes of the dorsal striatum receiving input from these areas. The rodent grooming behavior is organized into a number of discrete behavioral phases, each characterized by the execution of a very stereotypic motor program, which are combined into longer action sequences. As such, it is a good candidate for studying transitions in behavior.
Recordings of single neuron firing patterns in female Sprague Dawley rats revealed a strong engagement of these circuits in the on- and offset of grooming but also in the transitions between individual grooming phases. Based on our data we suggest a mechanistic model where corticostriatal circuits are controlling behavioral transitions through a population-gating mechanism involving both projection neurons and interneurons in cortex and striatum.
These results provide the first description of corticostriatal control mechanisms underlying behavioral transitions in a natural, complex and spontaneous behavior. The way these circuits control behavioral transitions may give important clues to the pathophysiological mechanisms underlying motor dysfunction in diseases involving cortico-basal ganglia circuits.

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Support: European Research Council (ERC-2013-CoG – 615699_NeuroKinematics, D.R.)

Title: Population coding of kinematic features in the striatum during motor learning

Authors: *W. TAOUALI[1], P. E. RUEDA-OROZCO[2], D. ROBBE[1];
[1]INSERM-INMED UMR901, Marseille Cedex 09, France; [2]Univ. Nacional Autónoma de México, Mexico City, Mexico

Abstract: Well-learned actions are typically executed with very little variability to comply with action-specific constraints. The neuronal mechanisms that contribute to constraining the trajectory and timing of learned movements are unclear. Recently a role of the dorsal striatum in controlling the kinematics of actions has been proposed and is supported by monotonic correlations between firing rate of individual neurons and movements kinematics. These correlations may indeed underlie neuronal encoding, defined here as the capacity to predict specific kinematic features from neuronal activity. However, monotonic regression analyses impose a very specific type of relationship between neuronal activity and the kinematics features to be decoded. In other brain regions such as the hippocampus strong encoding has been observed despite non-linear relationships between neuronal activity and the decoded feature. Moreover, how monotonic correlations between movements kinematics and neuronal firing rates translate into predictive power both at the single cell and populations level is not known. To address these issues, we applied a Bayesian decoder to spiking activity recorded in the dorsal striatum of rats running on a motorized treadmill. Two groups of rats were compared: well-trained animals performing a stereotyped running sequence and naive animals hand-guided to perform a similar running sequence. We first measured the accuracy of decoding the animals running speed, acceleration, position on the treadmill and time passing during the sequence. Decoding accuracy using single neurons was very low in both groups but increased with the number of neurons included in the decoder. Importantly, population decoding accuracy was higher in well-trained rats compared to naive rats. Notably acceleration and time, despite weak monotonic correlations with firing rates, were well decoded at the population level in the Bayesian framework. Finally, we report that the gain in decoding could be partially accounted for by a reduction of neuronal intertrial variability, but also by less expected changes in single cell tuning properties such as firing rate range. The contribution of these changes to the decoding depends on the decoded feature and the population size. These results suggest that the encoding accuracy of task-relevant kinematic features by single striatal neurons is generally weak and does not change after motor learning, despite a gain in monotonic correlations after learning. However, at the population level there is a better encoding of task-relevant kinematic features which can not be solely explained by a reduction of neuronal variability.

Disclosures: W. Taouali: None. **P.E. Rueda-Orozco:** None. **D. Robbe:** None.

Poster

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Title: Thalamic intralaminar nuclei control of striatal microcircuits

Authors: *U. GYAWALI, K. K. COVER, M. H. PATTON, B. M. ROBERTS, M. G. WHITE, B. N. MATHUR;
Pharmacol., Univ. of Maryland, Baltimore, Baltimore, MD

Abstract: Successful reward acquisition in a dynamic environment requires attention to salient sensory stimuli that instruct striatally-encoded actions. While striatally-projecting intralaminar nuclei of the thalamus encode salient information, how the different thalamic intralaminar nuclei govern striatal activity requires further investigation. To probe this, we employ optogenetics, electrophysiology and fast-scan cyclic voltammetry in mouse brain slices. Our preliminary data suggest that individual intralaminar nuclei form unique striatal microcircuits to differentially control striatal dopamine release. These results shed new light on the role of the thalamus in action control.

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Topic: E.03. Basal Ganglia

Title: Cell-type- and projection-specific study of the external globus pallidus circuit organization

Authors: *V. LILASCHAROEN, S. SHIN, D. A. KNOWLAND, B. LIM;
Biol. Sci., UCSD, La Jolla, CA

Abstract: Recent advances in circuit tracing have provided powerful tools that reveal extensive information about the neural circuitry. However, thus far, no viral tracer has been proven potent enough to allow a robust, long-lasting retrograde expression of transgenes without inducing cytotoxicity. Because even neighboring neurons of the same cell-type differ in their connectivity and functions, a new method is required to achieve both cell-type- and projection-specific delivery of transgene via retrograde transduction. Here we present a new viral-genetic strategy that allows long-term expression of transgenes including fluorescent proteins, optogenetic tools and genetically encoded calcium indicators in a cell-type- and projection-specific manner. We have developed a novel viral vector based on the rabies-glycoprotein-pseudotyped equine infectious anemia virus (RG-EIAV), which has been proven successful to infect neurons retrogradely. We genetically engineered the RG-EIAV vector to express site-specific recombinases including Flp and Dre in a Cre-dependent manner. Together with recombinant adeno-associated virus (AAV) expressing transgenes of interest in a Flp- or Dre-dependent manner, we are able to induce robust expression of transgenes in distinct neuronal populations of the same cell-type based on their projection targets. We demonstrate the application of this strategy by studying the circuit architecture of the parvalbumin-expressing neurons in the mouse external globus pallidus (GPe), one of the major nuclei of the basal ganglia that is thought to contribute prominently to basal ganglia dysfunction in movement disorders such as Parkinson's disease. The characterization of the GPe neuronal population including cell-types, circuit organization, and functional roles in pathological symptoms have not been fully addressed. By applying our novel strategy with transgenic mice expressing Cre recombinase in specific neuronal cell-types, we were able to identify two distinct populations of parvalbumin-expressing neurons in the GPe based on different projection patterns. This strategy will allow us to further our understanding of the GPe circuitry by elucidating efferent and afferent connections, electrophysiological properties and transcriptional profile of each specific neuronal population.

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Title: Theta and gamma rhythm generation in a model network of fast-spiking striatal interneurons

Authors: *J. A. CHARTOVE, M. MCCARTHY, B. PITTMAN-POLLETTA, N. KOPELL; Boston Univ., Boston, MA

Abstract: Theta (4-10 Hz) and gamma (40-90 Hz) oscillations in the striatum manifest during behaviorally relevant times in movement, reward, and decision making tasks, as does their cross-frequency coupling. Fast-spiking interneurons (FSIs) in striatum strongly inhibit the medium spiny neurons (MSNs), the output cells that make up 95% of striatum; therefore, they have a powerful influence in patterning striatal activity. These FSIs communicate with one another using both GABA-mediated synapses and dendro-dendritic gap junctions, and therefore comprise a coupled network potentially involved in producing behaviorally relevant rhythms within the striatum.
To explore rhythm generation by FSIs, we simulated a network of 100 fast-spiking interneurons, each of which was a two-compartment Hodgkin-Huxley neuron with a D-type slowly-inactivating potassium current (D-current). In isolation, these model neurons burst at 5 Hz when stimulated tonically, with spikes within a burst occurring at 75 Hz. These frequencies are determined by the time constants of the potassium currents within the cell. A network of these FSIs can produce either a theta frequency, a gamma frequency, or both, depending on the conductances of the gap junctions and inhibitory synapses between the individual cells. We simulated increased dopaminergic tone by increasing gap junction conductance as well as excitability of the FSIs, and decreasing D-current conductance. The rhythmic regime of the network was altered by changes in dopaminergic tone; our findings suggest that dopamine may induce the theta and high gamma (80 Hz) rhythms seen in vivo and carried by striatal FSI networks. Thus, theta and gamma rhythms in striatum and cortex may reflect dopaminergic tone, and may play a role in processing related to reward and motivation during decision making.

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CHDI

Title: Distinct firing patterns of GPe Arkypallidal and Prototypical neurons in a reinforcement learning task

Authors: *M. A. FARRIES[1,2], J. D. BERKE[1,2];
[1]Dept Psychology, Univ. of Michigan, Ann Arbor, MI; [2]Neurol., Univ. of California San Francisco, San Francisco, CA

Abstract: The basal ganglia (BG) are a set of subcortical structures involved in the modulation and control of adaptive behaviors. The GPe lies at the center of the BG network, receiving projections from input stations of the BG (striatum and subthalamic nucleus) and making GABAergic projections to all major BG nuclei. The GPe is best known for its projections downstream to BG output nuclei, but Mallet et al. (*Neuron* 2012) identified a distinct class of GPe cells, dubbed "Arkypallidal" (Arkys), that project exclusively to the striatum. Arkys can be distinguished from "Prototypical" (Proto) GPe cells by their firing rates and variability during natural waking and sleep states, and are especially responsive to Stop cues (Mallet et al. *Neuron 2016)*. To gain further insight into their distinct functions, we recorded presumed Arkys and Protos in rats engaged in a trial-and-error decision-making task (Hamid et al. *Nat Neurosci 2015).* On each trial, rats hold their nose in a central port until a Go cue prompts a left- or rightwards movement, with each option rewarded probabilistically. Unlike Protos, Arkys were generally more active during the intertrial interval (ITI), reducing their firing just before trial initiation and elevating it after trial completion. Both Arkys and Protos were more active, on average, when preparing contralateral movements compared to ipsilateral movements, but for Protos (only) this choice-related activity appeared even before the Go cue prompted movement. Both Arkys and Protos increased firing, on average, following reward delivery compared to unrewarded trials. However, this reward response was stronger and more consistent in Arkys, and the Arky population sustained reward-related changes in activity throughout the ITI, including an elevation in firing rate just before the onset of the next trial when the previous trial had been rewarded. We suggest that Protos are more concerned with choice preparation and execution, while Arkys are more concerned with trial outcomes, providing value information to the striatum during the ITI that may influence decisions to work.

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Poster

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Topic: E.03. Basal Ganglia

Title: Synaptic responses and plasticity properties in corticostriatal circuits *In vivo*

Authors: *K. JUCZEWSKI[1,2], D. A. KUPFERSCHMIDT[1], D. M. LOVINGER[1];
[1]Natl. Inst. on Alcohol Abuse and Alcoholism, Rockville, MD; [2]Dept. of Neurosci., Karolinska Inst., Stockholm, Sweden

Abstract: Corticostriatal circuits are involved in action control and action learning. The prefrontal cortex (PFC) projects strongly to the dorso-medial striatum (DMS) while the primary motor cortex (M1) projects mainly to the dorso-lateral striatum (DLS). It is believed that goal-directed actions are mostly controlled by DMS whereas habitual behaviors are controlled by the DLS. To understand specific dynamics of these circuits we used a classical physiological technique, local field potential (LFP) recording in urethane anaesthetized mice. In our studies we focused on several aspects of synaptic responses and plasticity evoked by cortical stimulation with a tungsten electrode and recorded by a glass pipette. Because little is known about origin and significance of the corticostriatal LFPs, we characterized these responses. We changed depth of the recording electrode, intensity of the stimulating current and stimulation parameters. We observed systematic changes in the waveforms of evoked LFPs that depended on all the mentioned parameters. Next, we combined electrical stimulation and optogenetics to take advantage of unique precision of this latter technique for pathway-specific interrogation. We determined that field potentials can be evoked with ChR2 expression and light stimulation. Mice expressing ChR2 in cortex were generated by breeding Emx1-Cre mice with AiCOP4 conditional ChR2-expressing mice. We performed in vivo recordings in DLS and DMS to compare electrically- and optically-evoked field potentials. We examined effects of stimulus duration and light power on the response characteristics. Additionally, light-evoked responses were compared in M1 and at different depths of the striatum showing topographical organization of this region. Control experiments indicated no light-evoked responses in C57Bl6/J mice that did not express ChR2. In ongoing experiments we are examining mechanisms of synaptic modulation and plasticity using various electrical- and light-stimulation patterns and intracerebroventricular drug application. Moreover, we are combining in vivo fiber photometry with our LFP-recording system that will give us a new insight into real-time plasticity changes observed in the corticostriatal circuits.

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Poster

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Topic: E.03. Basal Ganglia

Title: Motor cortex: a tutor for the basal ganglia?

Authors: *S. B. WOLFF, A. K. DHAWALE, R. KO, B. P. OLVECZKY;
Harvard Univ., Cambridge, MA

Abstract: The remarkable capacity of the brain to acquire and execute motor skills depends on a distributed motor network. While many components have been identified, the intricate interplay between the distinct brain areas involved, their specific roles, and the implementation of the learning process in neuronal circuitry remain elusive. To dissect how the motor system underlies the acquisition and execution of complex motor skills, we train rats in a lever-pressing task which results in spatiotemporally precise movement patterns. Previously, we addressed the role of motor cortex (MC), showing that the learned skills we train survive chronic lesions of MC, while transient optogenetic manipulations of MC acutely degrade their execution. Thus, while activity in MC has the capacity to modulate the learned skills, it seemingly does not exercise this function once the behavior has been consolidated. This is consistent with our previous finding that MC is necessary for the initial acquisition of the skills we train. Based on these results we hypothesize that MC acts as a 'tutor' during skill learning, with its input to downstream motor areas serving as a teaching signal that guides the acquisition and refinement of subcortically generated motor sequences. Here we show that the striatum, in particular the part that receives input from MC, is necessary for both acquiring and executing the motor skills we train, thus identifying striatum as a possible subcortical target of MC's tutoring function. To address the role of MC-striatum interactions directly, we use viral and molecular strategies to selectively access distinct MC projection pathways. In line with our hypothesis, transient optogenetic manipulations of corticostriatal projections acutely degrade the execution of the learned skills. Moreover, chronic and selective silencing of corticostriatal projections prevents animals from acquiring those skills in the first place. Together with our previous findings, this supports the idea of MC acting as a tutor of subcortical circuits through its modulation of striatal activity. To further dissect MC's tutoring role, we are using chronic long-term electrophysiological recordings and optogenetic identification of projection neurons during skill acquisition to study the activity of corticostriatal projections and the changes in cortical and striatal networks over the course of learning.

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Support: NKFI 109754

Title: Efficient cortical control of basal ganglia recipient motor thalamus.

Authors: *H. BOKOR, N. HADINGER, L. ACSADY;
Inst. of Exptl. Medicine, Hungarian Acad. of Sci., Budapest, Hungary

Abstract: The motor thalamic nuclei are key players in the circuit that control motor planning and execution. The thalamocortical neurons which receive GABAergic afferents from the output nuclei of basal ganglia (BG) project to frontal, motor cortical areas (M1, M2). Conversely layer 5 (L5) neurons of these regions innervate the first station of the BG circuit, the striatum, as well as its last station, the BG recipient thalamic nuclei. In the present study we aimed to elucidate the physiological and anatomical features of the L5 corticothalamic (CT) pathway to the motor thalamic nucleus ventromedialis (VM) and compare it to L5 input to sensory thalamus (nucleus posterior, Po) as well as to layer 6 (L6) projection to VM. We investigated the L5 CT pathway using Thy1-Chr2-EYFP and Rbp4- and the L6 CT pathway by the ntsr1-cre mice lines. Selective labeling of L5 axons revealed only small diameter (1-2 μm) terminals in the VM compared to the giant L5 driver terminals in the somatosensory Po (4-8 μm). The size range of L5 boutons in VM was close to that of the L6 inputs (0.5-1μm). At the ultrastructural level, however, L5 and L6 terminals displayed significant differences in VM. L5 boutons established much larger, often multiple perforated or branching synapses, always contained mitochondria and had significantly larger volume. L5 terminals mostly innervated specialized large headed (>300nm) dendritic spines. In contrast, L6 terminals established simple and smaller synapses, targeted thin (≤0,5 μm) dendritic shafts and contained mitochondria only in 10% of the cases. Photoactivation of L5 input from M1 and M2 motor cortex could reliably evoke action potentials in VM cells under ketamine/xylazine anesthesia. Repetitive activation of L5 was able to entrain VM neurons firing. Several VM cells could be reliably driven with up to 20 Hz stimulation. In contrast, photoactivation of Po cells via the large S1, L5 terminals led to significant depression in response probability above 4 Hz. Unexpectedly, we found that VM cells can be driven by L5 input arising from the contralateral cortex as well. At the electron microscopic level L5 terminals from the contralateral cortex had larger volume and postsynaptic density area compared to the L5 terminals from the ipsilateral cortex. Axonal labeling and photoactivation of L5 neurons in different frontal cortical regions revealed that L5 afferents are organized according to horizontal sectors within VM.

Our results demonstrate that neocortex can exert strong, top-down influence on the information transfer at the last, thalamic stage of the basal ganglia circuit via morphologically specialized synaptic connections.

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Title: Communication between subthalamic structures and pontine nuclei

Authors: ***K. B. RAMAKRISHNAN**, K. KHODAKHAH;
Albert Einstein Col. of Med., Bronx, NY

Abstract: The subthalamic nucleus (STN), zona incerta (ZI), and pontine nuclei (PN) are subcortical brain regions that contribute to motor and non-motor related activity. The STN is part of the basal ganglia structure with primarily glutamatergic projection neurons. Although the projection of the STN within the BG circuit is well studied, its projection outside the BG is not well understood. The ZI, located dorsal to the STN, is known to send both excitatory and inhibitory projections to PN, which is part of brainstem. The PN, which also receives extensive cerebral inputs, targets the granular layer of the cerebellum as glutamatergic mossy fibers. These structures are all known to be involved in motor related activity, and their dysfunction may contribute to movement disorders.We examined whether STN or ZI affect the activity of the PN neurons by combining optogenetics with single unit recordings in awake mice. Channelrhodopsin was injected in either the STN or the ZI, and their axonal projections were optically activated in PN while recording the single cell activity of neurons using an optrode. We find that in STN-targeted mice, about 50% of recorded cells responded to the stimuli, whereas in ZI-targeted mice more than 75% of the recorded cells were responsive to optical excitation of the fibers. In both STN- and ZI- ChR2 mice, optical activation of the opsin carrying axons increased the firing rate in the PN neurons with a short latency of approximately 2-4 milliseconds. The stimuli also inhibited the spontaneous firing in about 20% of neurons. Our data suggest that both STN and ZI are capable of modulating the activity of the PN.

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Title: The basal ganglia and cerebellum contribute to the control of the adrenal medulla

Authors: *R. P. DUM[1], M. E. MARCELLE[2], D. J. LEVINTHAL[3], P. L. STRICK, 15261[2]; [1]Neurobiology, Ctr. for Neural Basis of Cognition, Systems Neurosci. Inst., [2]Neurobiology, Ctr. for Neural Basis of Cogn., Systems Neurosci. Inst., Univ. of Pittsburgh Brain Inst., Univ. Pittsburgh, Pittsburgh, PA; [3]Medicine, Div. Gastroenterology, Hepatology, and Nutr, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: We used retrograde transneuronal transport of rabies virus to determine if the adrenal medulla is the target of output from the basal ganglia and cerebellum. We injected rabies virus (N2c strain) into the adrenal medulla of 5 cebus monkeys. The animals were allowed to survive long enough to label second-order (n=1), third-order (n=1) or fourth-order neurons (n=3). We first observed substantial numbers of labeled neurons in the basal ganglia and cerebellum in animals with fourth-order labeling. This is the same survival time in which substantial numbers of labeled neurons were first present in the cerebral cortex. These observations indicate that the basal ganglia and cerebellar labeling was mediated by connections to efferent systems in the hypothalamus, midbrain or brainstem, rather than through the classical basal ganglia-thalamo-cortical or cerebello-thalamo-cortical circuits. The labeled neurons in the basal ganglia and cerebellum were consistently observed in the substantia nigra pars reticulata (SNpr), the internal segment of the globus pallidus (GPi) and each of the three deep cerebellar nuclei. Overall, we observed about four times as many labeled neurons in the basal ganglia as in the cerebellum. Within the basal ganglia, most labeled neurons (80%) were located in the SNpr and were concentrated in a subregion that is interconnected with area 9 in the prefrontal cortex. The remainder of basal ganglia labeling was scattered throughout the motor and non-motor regions of the GPi. Within the cerebellum, more than half of the labeled neurons were located within the ipsilateral (30%) and contralateral (21%) fastigial nucleus. These results indicate that the basal ganglia and cerebellum can influence at least one element of the sympathetic nervous system- the

adrenal medulla. This influence appears arise more prominently from the basal ganglia than from the cerebellum. Moreover, this basal ganglia output originates primarily from its non-motor region. Our results suggest that the cerebellum, and especially the basal ganglia, are involved in the neural regulation of internal organ function. Thus, our anatomical results may provide a neural substrate for autonomic dysfunction that is associated with basal ganglia and cerebellar disorders.

Disclosures: R.P. Dum: None. **M.E. Marcelle:** None. **D.J. Levinthal:** None. **P.L. Strick:** None.

Poster

720. Physiology of Basal Ganglia Systems

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 720.21/SS19

Topic: E.03. Basal Ganglia

Support: R25GM069621-11

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8G12MD007592

2G12MD007592

Title: Glycinergic neurons are implicated in basal ganglia dependent functions

Authors: *Y. P. HUIZAR[1], R. ORTEGA[2], R. A. PEREZ[2], E. CASTANEDA[3], M. MIRANDA[2]; [1]Biochem., Univ. of Texas At El Paso, El Paso, TX; [2]Biochem., [3]Psychology, Univ. of Texas at El Paso, El Paso, TX

Abstract: The basal ganglia is responsible for somatosensory discrimination and both voluntary and involuntary movement. The functions regulated by the basal ganglia involve the release of dopamine from dopaminergic nerve terminals in the striatum. Activation of post-synaptic dopamine receptors leads to activation of the direct and indirect pathways to regulate motor function. Although the contribution of GABAergic fibers to basal ganglia function is well documented, glycinergic neurotransmission is completely unknown. We are currently analyzing glycine transporter 1 (GlyT1) immunoreactivity in the globus pallidus and our findings suggest the presence of GlyT1 in neurons. To investigate the functions regulated by these glycinergic neurons, sprague dawley rats were subjected to intracranial injections of saline or the glycine receptor antagonist strychnine followed by motor assessment, sensorimotor evaluation, testing

postural reflexes, and analyzing open-field behavior. The results suggested a potential contribution of glycinergic transmission in the regulating voluntary motor activity. Current and future research will elucidate the role of nigropallidal glycinergic fibers in the control of motor functions.

Disclosures: Y.P. Huizar: None. **R. Ortega:** None. **R.A. Perez:** None. **E. Castaneda:** None. **M. Miranda:** None.

Poster

720. Physiology of Basal Ganglia Systems

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Program#/Poster#: 720.22/SS20

Topic: E.03. Basal Ganglia

Title: Optogenetic interrogration of distinct and complementary nigral sub-circuts that shape movement.

Authors: *G. I. RIZZI[1], K. R. TAN[2];
[1]Biozentrum, [2]Univ. of Basel, Basel, Switzerland

Abstract: Motor behavior stands at the core of every mammalian organism's survival and evolvement within its environment. The Substantia Nigra Pars Reticulata (SNr) has long been identified as the prime output nucleus of the Basal Ganglia, an ensemble of brain structures that governs motor behavior. The SNr is commonly regarded as GABAergic and homogenous. Its activity patterns have been correlated with different aspects of locomotion such as sensory input, movement planning, execution and interruption. Such versatile function suggests a more complex organization of the SNr than has been thought so far. Using a combination of anatomical and functional techniques we identified two marginally overlapping sub-populations of GABAergic neurons that are embedded within separate circuits, execute different functions, and act complementarily to warrant locomotion. More specifically, we used optogenetic-targeting techniques to dissect the function of each nigral sub-population and verify the effect of their combined interaction. While VGAT-positive neurons encode the execution of movement, PV-expressing cells alone do not alter locomotion. Interestingly when activated synchronously, the two populations drastically increase the robustness of locomotion.
We provide fundamental understanding of basic SNr circuitry and function with a cell-type specific resolution. This represents a critical step in refining our knowledge in the cellular, synaptic and circuit bases of motor-related behaviors.

Disclosures: G.I. Rizzi: None. **K.R. Tan:** None.

Poster

720. Physiology of Basal Ganglia Systems

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Program#/Poster#: 720.23/SS21

Topic: E.03. Basal Ganglia

Support: SNSF

Title: The motor cortex to substantia nigra: a novel pathway

Authors: *M. LODGE, G. RIZZI, K. R. TAN;
Biozentrum, Basel, Switzerland

Abstract: The basal ganglia, a complex network of interconnected nuclei, have been linked to the formation and execution of voluntary movement. The principal model of movement control involves two distinct pathways originating from direct spiny projection neurons (dSPNs) and indirect projection neurons (iSPNs) of the striatum. Through different projection targets the dSPNs and iSPNs are able to initiate and stop movement respectively, via the substantia nigra pars reticulata (SNr). Here, we describe a novel pathway from the motor cortex directly onto the SNr, and we aim to elucidate the role of this pathway in locomotion.
Using retrograde and anterograde mapping tools we show an anatomical connection between the M1/M2 motor cortex and the SN. Functional connectivity is investigated using *in vitro* electrophysiology, providing confirmation of a glutamatergic projection onto two cell populations- IH positive (putative dopamine neurons) and IH negative (putative GABA neurons)- with a preferential connection to IH positive neurons. To expand on this we will use optogenetics in combination with a motor task to investigate its role in motor behaviour output. Thus, we hope to understand what this novel pathway does in relation to the currently accepted roles of the direct and indirect pathways- and more importantly how this opens the door to more complex locomotor circuitry.

Disclosures: **M. Lodge:** A. Employment/Salary (full or part-time): University of Basel. **G. Rizzi:** A. Employment/Salary (full or part-time): University of Basel. **K.R. Tan:** A. Employment/Salary (full or part-time): University of Basel.

Poster

720. Physiology of Basal Ganglia Systems

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 720.24/SS22

Topic: E.03. Basal Ganglia

Support: CHDI Foundation #A-8427

Title: Reward based motor adaptation mediated by basal ganglia

Authors: *T. KIM[1], K. C. HAMADE[1], W. TEKA[2], W. H. BARNETT[2], S. N. MARKIN[1], I. A. RYBAK[1], Y. I. MOLKOV[2];
[1]Drexel Univ. Col. of Med., Philadelphia, PA; [2]Georgia State Univ., Atlanta, GA

Abstract: The basal ganglia (BG) are involved in a broad range of motor and cognitive behaviors. It is widely accepted that the BG play a key role in action selection and reinforcement learning facilitated by the activity of dopaminergic neurons that encode the prediction error when the reward outcome is higher or lower than expected. The BG are thought to select proper motor actions by gating appropriate commands and suppressing inappropriate ones from a repertoire of cortical signals arriving at the striatum. This process of action selection is mediated by the direct striato-nigral (GO), and the indirect stiato-pallidal (NOGO) pathways. Previous models confirmed the plausibility of the above concept, but in an abstract way only, lacking the details about formation of neuronal activity patterns actuating the muscular-skeletal apparatus, and hence did not allow studying BG involvement in motor adaptation. We have developed a more complete and detailed model of BG interacting with a movement execution system in the context of goal directed reaching tasks. The model included sensory and motor cortices, the BG, the spinal cord circuitry, and a virtual mechanical arm allowing for simulation of 2D reaching movements. The arm was composed of 2 joints (shoulder and elbow) controlled by 6 muscles (4 mono-articular and 2 bi-articular). The spinal cord network contained motoneurons to control 6 muscles and sensory interneurons receiving afferent feedbacks and providing basic reflexes. The cortical neural activity corresponding to possible movements was determined by solving an inverse problem based on the initial arm position, reaching distance and direction. Reaching movements were performed in response to a sensory cue associated with the target arm position. Target positions located in an arbitrary number of different directions were used as a set of possible actions. The function of BG was to associate the cue with a proper reaching movement by adjusting weights of cortico-striatal inputs through reinforcement learning. The BG had an intrinsic exploratory mechanism allowing for seeking better cue-action responses delivering larger rewards. Reinforcement learning relied on the dopaminergic signal measuring trial-to-trial changes in the reward. The model was used to simulate reward based adjustment of reaching movements to verify that such a system is capable of quick (10-100 trials) motor adaptation

observed in experiments. Our analysis shows that (1) potentiation of cue-NOGO inputs is crucial for quick dropping of pre-existing cue-action associations; (2) quick learning occurs at the expense of relatively poor accuracy and high variability of the movements.

Disclosures: **T. Kim:** None. **K.C. Hamade:** None. **W. Teka:** None. **W.H. Barnett:** None. **S.N. Markin:** None. **I.A. Rybak:** None. **Y.I. Molkov:** None.

Poster

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Topic: E.03. Basal Ganglia

Support: CAS Grant Y51HN44541

Title: The control of action selection by cell-type specific circuits of the striatum in auditory-guided decision task

Authors: ***L. CUI**[1,2], N. XU[1];
[1]Inst. of Neuroscience,CAS, Shanghai City, China; [2]Univ. of Chinese Acad. of Sci., Shanghai, China

Abstract: The transformation from sensory information to motor actions is a fundamental process in decision making. Although the striatum has been implicated in movement control and decision making, its critical role in the sensory-motor decision is still elusive. This can be due to the lack of proper quantitative behavioral measurement with correspondence to precise manipulation of specific striatal circuits, where different sub-regions and different output (direct and indirect) pathways of the striatum could play distinct roles. Here we combined quantitative psychophysics task in mice with specific circuit manipulations to address this problem. We trained head-fixed mice to perform an auditory-guided two-alternative forced choice (2AFC) task, in which mice distinguished sounds with different frequencies and licked left or right water port to report their choice. This behavior paradigm provided precise time control and accurate behavioral readout, allowing for probing the striatal circuits in different aspects of sensory-based action selection. First, we examined the regional specific roles of striatum in the auditory-guided action selection. Unilateral optogenetic silencing of a posterior sub-region of the dorsal striatum, where it receives input from auditory cortex, impaired mice's contralateral choice but not the ipsilateral choice, whereas silencing of an anterior region of dorsal striatum, where it mainly receives input from motor cortex, showed no significant effect on behavioral performance, suggesting a region and modality specific functional role of the striatum in action selection.

Second, we specifically manipulated subtypes of striatal projection neurons associated with direct or indirect output pathways. Unilateral activation of D2 receptor expressing neurons (using floxed-ChR2 in D2-cre mice) suppressed contralateral behavioral choice, suggesting a suppressive role of the indirect pathway in action selection. In contrast, activation of D1 receptor expressing neurons (using floxed-ChR2 in D1-cre mice) drove the contralateral choice, supporting sufficiency of the direct pathway in driving contralateral choices. Taken together, our results provide direct evidence for the control of action selection in perceptual decision-making by sub-region and cell-type specific circuits in the striatum. Prospectively, our behavior paradigm, when combined with optogenetics and in vivo imaging techniques, will provide a unique window to gain mechanistic understanding of the operation principles of basal ganglia circuits in sensory-motor decision making.

Disclosures: L. Cui: None. **N. Xu:** None.

Poster

720. Physiology of Basal Ganglia Systems

Location: Halls B-H

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Topic: E.03. Basal Ganglia

Support: JSPS-16H03200

Title: An Influence of motor costs in human reinforcement learning

Authors: *K. SATO, J. IZAWA;
Empowerment Infromatics, Univ. of Tsukuba, Tsukuba, Japan

Abstract: The principle of the motor learning is forming an association between sensory observation and motor actions: a motor policy of how the brain select optimal actions at the certain state of the body and the environment. There is ample evidence to suggest that the brain employs optimal feedback control in computing motor commands that maximize reward and minimizes effort (i.e. motor costs) [Izawa 2008, 2011]. Yet, how exactly the brain optimizes the motor policy is still unclear. On the other hand, the reinforcement learning that fits well with the behavioral data of decision making tasks has uncovered the roles of the basal ganglia in maximizing future rewards with exploration and exploitation of actions, which is mathematically identical to an optimization algorithm of forming the state-action map whereas its role in human motor control has not been well examined. To seek a contribution of reinforcement learning mechanism of the human brain to optimization of motor commands, we focused on the motor costs in a human reinforcement learning task and investigated the property of the brain in

forming motor policy over the visual state space which was also influenced by the physical effort of actions.
We extended the 'grid sailing task' developed by Doya and his colleagues [Fermin 2010] where the subjects were asked to explore an optimal route of the agent's (i.e. visual cursor) navigation from the start cell to the goal cell by deciding the agent's action with pushing the keys: This is similar to learning for controlling a new tool with finding a series of the finger tapping although a computational problem behind this task is forming the state-action pairs rather than memorizing a tapping sequence. Based on this paradigm, we simplified the state space and implemented the motor cost: The subjects pushed the two force sensors with the index and the middle fingers instead of a keyboard, where we manipulated the amount of the force that the subjects were requested to produce to select agent's actions which brought the cursor to the next state.
We observed a route selection difference between the cost situation and no-cost one: The selected action at each state is biased to one with the lower motor cost while maintaining the low number of actions. Also, we observed that subjects generalized the previously learned state-action memory among two situations with different motor costs. This result suggests that the human brain has the capacity to learn state-action pairs by taking account of the physical efforts to achieve the goal. The further analysis with Q-leaning model may reveal the structures of the action-value memories for the reward-based and the effort-based motor optimization.

Disclosures: K. Sato: None. **J. Izawa:** None.

Poster

720. Physiology of Basal Ganglia Systems

Location: Halls B-H

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Topic: E.03. Basal Ganglia

Support: Canadian Institutes of Health Research

Title: Distinct roles of subthalamic nucleus alpha and beta oscillations in stopping and delaying manual and verbal actions

Authors: *A. GHAHREMANI[1,2], K. UDUPA[3], J. WESSEL[4], U. SAHA[3], T. HOQUE[3], A. LOZANO[3], M. HODAIE[3], S. KALIA[3], A. ARON[5], R. CHEN[3];
[1]Toronto Western Hosp., Toronto, ON, Canada; [2]Univ. of Toronto, Toronto, ON, Canada; [3]Krembil Neurosci. Ctr., Toronto, ON, Canada; [4]Univ. of Iowa, Iowa, IA; [5]Univ. of San Diego, San Diego, CA

Abstract: Inhibitory control over the motor system is thought to be implemented by a frontal-basal ganglia network to either delay or entirely stop actions. The subthalamic nucleus (STN) of the basal ganglia is considered a key node in this network. Several studies of manual stop tasks have reported increased power of STN beta band [13-30Hz] oscillations when actions are successfully stopped. Studies with other response control tasks reported increased STN power in lower frequencies such as alpha [8-12Hz] and theta [4-8Hz] bands in relation to delaying responses. Here we recorded oscillations from the STN in two groups of Parkinson's disease patients undergoing deep brain stimulation surgeries. We ran a standard manual stop signal task (n=8) and a speech stop signal task (n=10). Our objectives were to compare manual and verbal versions with respect to STN signatures for a) stopping (i.e. successful vs. failed stop trials) and b) delaying movement (i.e. going slow vs. fast on Go trials). Consistent with several studies, we found increased beta activity in the STN for successfully stopping manual actions ($F(1,7)=12.27$, $p=0.01$). For the comparison of slow "go" trials compared to fast "go" trials, alpha activity was commonly increased in the STN in both manual and speech tasks (manual: $t(7)=5.66$, $p=0.002$; speech: $t(9)=4.51$, $p=0.003$). Thus, increased oscillatory power was detected in the STN for inhibitory control of both manual and verbal actions. The findings also show that different signatures (beta vs. alpha) relate to stopping vs. delaying actions, possibly related to different frontal-STN hyperdirect pathways.

Disclosures: **A. Ghahremani:** None. **K. Udupa:** None. **J. Wessel:** None. **U. Saha:** None. **T. Hoque:** None. **A. Lozano:** None. **M. Hodaie:** None. **S. Kalia:** None. **A. Aron:** None. **R. Chen:** None.

Poster

720. Physiology of Basal Ganglia Systems

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Topic: E.03. Basal Ganglia

Support: CIHR (MOP102662)

CFI

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GRSNC

Title: The basal ganglia control the urgency of a reach choice, but not the choice itself

Authors: *D. THURA, P. CISEK;
Dept Neurosci., Univ. Montreal, Montreal, QC, Canada

Abstract: The basal ganglia (BG) have long been implicated in many aspects of cognition and motor control. One prominent theory suggests that they play a key role in selecting desired actions and suppressing competing actions on the basis of decision-variables learned through reinforcement. However, choice selective signals in the GPi often lag behind cortical regions and are largely simultaneous with muscle contraction. Furthermore, GPi inactivation has no effect on which movements are selected in decision tasks, but instead reduces the velocity and extent of movements, consistent with the proposal that the BG regulate motivation and response vigor. It therefore remains unclear whether the BG play a causal role in decision-making and if so, precisely what that role may be. To investigate this question, we recorded cortical and pallidal activity in two monkeys trained to perform a reach selection task that allows us to dissociate the process of deliberation from the moment of commitment. In different blocks, the temporal properties of the task were varied to induce adjustments of monkeys' speed-accuracy trade-off (SAT). We found that while animals deliberate between two reach targets, neurons in dorsal premotor (PMd) and primary motor cortex (M1) continuously reflect the evolving sensory evidence guiding the decision. By contrast, BG output via the globus pallidus internus (GPi) remains untuned until the commitment time is signaled in PMd/M1, possibly confirming the cortical decision and movement initiation. We also observed that the SAT context in which the task was executed strongly modulated a large proportion of GPe (50%) and GPi (63%) cells. Crucially, this modulation was congruent with the cells' variation of activity during deliberation within each block: "build-up" cells tended to be *more* active during fast than slow blocks, especially in GPe; while "decreasing" cells were *less* active in fast than slow blocks, suggesting that the BG provides a time-varying signal reflecting the growing "urgency" to commit, which is adjusted to control speed/accuracy tradeoffs. Our results are consistent with the proposal that cortical activity reflects a dynamic, biased competition between candidate actions, which is gradually amplified by an urgency signal from the BG that effectively controls the amount of evidence needed before the animal commits to the currently favored reach choice. Eventually, the cortical bias in favor of one of the targets becomes strong enough to engage tuning in the GPi, at which point the BG "gate" is opened leading to a positive feedback that constitutes commitment to the action choice.

Disclosures: D. Thura: None. **P. Cisek:** None.

Poster

720. Physiology of Basal Ganglia Systems

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Topic: E.03. Basal Ganglia

Support: NSERC PDF 438487-13

Title: Real-time *In vivo* plasticity of corticostriatal afferent activity during skill learning

Authors: *D. A. KUPFERSCHMIDT[1], G. CUI[2], K. JUCZEWSKI[3,4], D. LOVINGER[3];
[1]Lab. for Integrative Neurosci., Natl. Inst. on Alcohol Abuse and Alcoholism, Rockville, MD; [2]Natl. Inst. on Envrn. Hlth. Sci., Research Triangle Park, NC; [3]Lab. for Integrative Neurosci., Natl. Inst. on Alcohol Abuse and Alcoholism, Rockville, MD; [4]Dept. of Neuroscience, Karolinska Inst., Stockholm, Sweden

Abstract: Dynamic changes in cortico-basal ganglia circuit function underlie action learning. How functional changes in discrete, connectivity-specified circuits manifest in vivo during such learning remains unknown. Using in vivo fiber photometry, we assessed real-time activity and plasticity of distinct cortical inputs to the striatum during motor skill learning. The genetically encoded calcium indicator, GCaMP6s, was virally expressed in excitatory cortical neurons in medial prefrontal cortex (mPFC) or motor cortex (M1) of mice. An optical fiber was implanted into dorsomedial striatum (DMS) of mPFC-injected mice to target associative inputs, and dorsolateral striatum (DLS) of M1-injected mice to target sensorimotor inputs. Activity-dependent fluorescent calcium dynamics were assessed in presynaptic elements of these inputs as a proxy for projection activity as mice were trained on the accelerating rotarod. Engagement of associative inputs was modest during initial trials on the rotarod, peaked during early training, and rapidly diminished as performance was automatized. In contrast, sensorimotor inputs were robustly engaged during initial trials, and showed a progressive decrease in activity with training. Somatic photometric recordings of DMS-projecting mPFC neurons and DLS-projecting M1 neurons revealed unique engagement patterns, suggesting differential learning-related plasticity at somatic and presynaptic elements of corticostriatal inputs. Our work describes novel approaches to observe real-time activity dynamics in discrete corticostriatal inputs, and provides new insight into how cortico-basal ganglia circuits encode action learning.

Disclosures: D.A. Kupferschmidt: None. **G. Cui:** None. **K. Juczewski:** None. **D. Lovinger:** None.

Poster

720. Physiology of Basal Ganglia Systems

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Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 720.30/TT2

Topic: E.03. Basal Ganglia

Support: Stanford Neurosciences Institute Interdisciplinary Scholar Award

Title: A role for dynorphin in the basal ganglia

Authors: *R. LALCHANDANI, J. B. DING;
Neurosurg., Stanford Univ., Palo Alto, CA

Abstract: The human brain has evolved to reward specific behaviors that increase the probability of propagating of one's genetic material. Drugs of abuse— such as cocaine, prescription painkillers and heroin— hijack these built-in systems to change the way different areas of the brain communicate. This shortcut to reward is especially problematic when drug experiences are linked to the memory of the circumstances in which drug use occurred, which often leads to relapse after periods of abstinence. Much remains unknown about how changes in reward circuits lead to addiction and how the brain creates memories that increase susceptibility to relapse. The basal ganglia are highly implicated in conscious cognitive processes, including learning, memory and addiction. The largest nucleus of the basal ganglia, the striatum, utilizes endogenous opioids to modulate information integration. Dynorphin is a potent opioid that is synthesized in striatonigral pathway neurons and is understood to fine-tune basal ganglia transmission at the synaptic level. Dynorphins act primarily on kappa-opioid receptors, which occur in high densities in the basal ganglia at both pre-synaptic and post-synaptic sites, and action of dynorphin at kappa-opioid receptors reduces the release of dopamine. Drugs of abuse cause increases in dynorphin expression in the basal ganglia, and the opiate antagonist, naltrexone, acts primarily at mu and kappa opioid receptors as a treatment against relapse in certain addictions. Despite the prevalence of the dynorphin in the basal ganglia and its link to addiction, its role in the basal ganglia is not well understood. As dynorphin is expressed widely throughout the brain, we have developed a system in which dynorphin can be removed in a cell-type specific manner and have subsequently assessed behavioral changes in response to selective dynorphin deletion.

Disclosures: R. Lalchandani: None. **J.B. Ding:** None.

Poster

721. Motor Systems: Oral Motor and Speech

Location: Halls B-H

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Topic: E.04. Voluntary Movements

Support: NIH-NIDCD Grant R01DC012502

NSERC-Canada

Title: Exogenous and endogenous auditory prediction in speech

Authors: *D. SHILLER[1], M. SATO[2];
[1]Ecole d'orthophonie et d'audiologie, Univ. of Montreal, Montreal, QC, Canada; [2]Lab. Parole et Langage, Aix-Marseille Univ. & CNRS, Aix-en-Provence, France

Abstract: Auditory sensory prediction plays a central role in speech motor planning and control. One approach to investigating this predictive process is through the comparison of auditory cortical responses to auditory feedback during active speech vs. passive listening to the same acoustic speech signals. Neural responses during active speech production are typically suppressed, which is presumed to reflect a subtraction of the motor-sensory prediction from auditory feedback. In the present EEG study, we further explored the link between online (feedback) and offline (passive listening) speech processing by measuring auditory evoked responses in combination with two different manipulations that alter a talker's ability to accurately predict the sensory consequences of speech actions: one involving a real-time change in auditory feedback during vowel production, and the other involving the presenting a stable auditory vowel target before each production.
Methods: Participants carried out two pairs of tasks, each consisting of matched speech production and passive listening phases. In each task pair, subjects first produced 240 vowel-consonant syllables during a portion of which vowel F1 frequency was altered in real-time (35% increase). Following production, subjects passively listened to the recorded speech sequence (identical in timing and amplitude to the preceding speech auditory feedback). Two such pairs of production/listening tasks were carried out, one involving a visual cue to speak and the other involving an auditory presentation of the speech target. During all tasks, averaged EEG auditory-evoked potentials (N1/P2) were computed for each task, feedback condition and target cueing condition.
Results: Behaviorally, subjects compensated for the auditory feedback manipulation under both visual and auditory-cueing conditions. EEG analyses showed a significant speech-induced suppression (SIS) of the auditory evoked N1/P2, with a difference in amplitude between the auditory and visually cued productions. Interestingly, this difference in SIS magnitude resulted from a differential effect of cueing modality on the evoked responses during the listening task,

with a reduction in auditory evoked N1/P2 between the auditory target and the subject's speech production (auditory habituation) occurring during offline listening, but not during online production.
In demonstrating distinct exogenous and endogenous predictive processes during speaking and listening, the results suggest that online auditory processing of speech feedback is functionally decoupled from offline processing of speech originating from outside the speaker.

Disclosures: D. Shiller: None. **M. Sato:** None.

Poster

721. Motor Systems: Oral Motor and Speech

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Topic: E.04. Voluntary Movements

Support: Grant-in-Aid for Scientific Research, KAKEN

Title: Cortical control of postural responses on swallowing movement: A fNIRS study

Authors: *M. YAMAWAKI, S. SHIBANO, G. MATSUDA;
Kyoto Prefectural Univ. of Med., Kyoto, Japan

Abstract: (Backgrounds) The swallowing center in the medulla is the key integrator of swallowing performance. In addition, there are subcortical and cortical centers above the brainstem that induced swallowing movement, however, their specific role and connections are not well understood. There are technical limitations of conventional neuroimaging techniques that require subjects to be in a supine position and/or restrict head movements. Such limitations narrow the range of experimental task options for swallowing. To challenge these issues, we applied functional near-infrared spectroscopy (fNIRS), an optical method that noninvasively measure cortical hemodynamics, for brain mapping in swallowing.
(Methods) Eighteen of right-handed healthy male were analysed. Subjects, on the chair or in supine position were put 34-channel holder of OMM-2000 Optical Multichannel Monitor (Shimadzu, Kyoto, Japan). An increase in oxyHb is used as an indicator for brain activation. Sensorimotor cortex and frontal lobe were set as the region of the interest. Data analysis was performed according to our previous study. Optode positions were measured using a 3D magnetic space digitizer (FASTRAK-Polhemus, Colchester, VT). Probabilistic method was used to register NIRS data to MNI (Montreal Neurological Institute) standard brain space. The statistical method used was a general linear model employing a two-level summary statistics approach for random effects analysis with a one-tailed t test.

(Results) Activation areas in each task were detected separately in SMI ($p < o.o5$ by Student t, one-tailed, FDR controlled). During swallowingin supine, activation was detected in tongue SMI and BA 40 ($p < o.o5$, one-tailed, FDR controlled). The haemodynamic pattern observed during swallowing was different in sitting versus supine position in BA 6 and BA 40 ($p < o.o5$, one-tailed, FDR controlled).
(Conclusions) The haemodynamic pattern during swallowing appeared different in sitting versus in supine position in BA 6 and BA 40. Our findings suggest that the sensory input is more important in supine than in sitting posture. Since fNIRS measurements are limited to the cortical surface, determining cortical connections to insula and basal ganglia in swallowing requires continued research.

Disclosures: **M. Yamawaki:** None. **S. Shibano:** None. **G. Matsuda:** None.

Poster

721. Motor Systems: Oral Motor and Speech

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Program#/Poster#: 721.03/TT5

Topic: E.04. Voluntary Movements

Title: Volitional vocal responses to unexpected perturbations in voice pitch auditory feedback

Authors: *C. R. LARSON[1], J. H. KIM[1], D. ROBIN[2];
[1]Communication Sci. and Disorders, Northwestern Univ., Evanston, IL; [2]Communication Sci. and Disorders, Univ. of New Hampshire, Durham, NH

Abstract: When monitoring voice pitch auditory feedback and controlling voice fundamental frequency (F0), speakers may adjust F0 to follow the change in feedback (feedforward) or to counter the change (feedback) for a correction. The present study measured neural responses (ERPs) from EEG electrodes as subjects vocalized and changed their F0 to either mimic the direction of positive or negative changes in the pitch feedback (follow up or down) or to oppose the directional change in feedback. The follow condition is similar to a feedforward control system, and the oppose condition is similar to a negative feedback control system. ERPs were obtained from a 32 channel EEG cap (BrainVision) as subjects vocalized into a microphone. Voice auditory feedback was presented to the subjects over earphones. Voice pitch feedback was altered at random times during the vocal tasks. In the feedforward condition, subjects were instructed to change their F0 in the same direction (up or down) as a change in voice pitch auditory feedback. In the feedback condition, subjects changed their voice F0 in the opposite direction to the change in voice pitch feedback. That is, if the feedback pitch increased, subjects decreased their F0. The N1 ERPs in the posterior electrodes (parietal areas) had significantly

(RM ANOVA, p< .000) shorter latencies for both feedforward (86 ms vs. 103 ms) and feedback (87.8 vs. 101 ms) responses than the the frontal electrodes. The P2 electrodes had significantly (p<.009) larger response magnitudes in the posterior electrodes for both the feedforward (2.25 vs. 1.05 uV) and feedback (2.15 vs. 0.86 uV)responses. The P2 ERPs also had significantly (p < .04) larger magnitudes in the left hemisphere compared to the right (2.25 vs. 1.48uV). Results of this study indicate left posterior regions of the brain, such as the left posterior temporal and parietal lobes may play important roles in volitional vocal responses to changes in voice auditory feedback.

Disclosures: C.R. Larson: None. **J.H. Kim:** None. **D. Robin:** None.

Poster

721. Motor Systems: Oral Motor and Speech

Location: Halls B-H

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Topic: E.04. Voluntary Movements

Support: This work was supported by the National Institute on Deafness and Other Communication Disorders, National Institutes of Health (R01DC01805 to KS)

Title: The role of the insula in speech motor control: structural connectivity profiles in healthy volunteers and spasmodic dysphonia patients

Authors: *G. BATTISTELLA[1], V. KUMAR[1], K. SIMONYAN[2];
[1]Dept. of Neurol., Icahn Sch. of Med. at Mount Sinai, New York, NY; [2]Dept. of Neurol. and Otolaryngology, Icahn Sch. of Med. At Mount Sinai, New York, NY

Abstract: The importance of the insula in speech control is acknowledged but poorly understood, partly due to a variety of clinical symptoms resulting from insults to its different regions. We conducted detailed examination of insular structural connectivity related to speech control in 12 healthy subjects (55±7 years, 7F/5M) and 12 patients with spasmodic dysphonia (SD, 54.1±11, 7F/5M), which is an isolated focal dystonia that selectively affects speech production. We used probabilistic diffusion tractography to parcellate the insula based on its structural connections with the Broca's area, Wernicke's area, and laryngeal motor cortex (LMC) and compared the anatomical distributions of the resulting partitions in HV and SD groups. We further used a 112-region whole-brain parcellation, including the parcellated insular regions identified above, to assess the insular large-scale structural network in HV and SD groups.
In HV, insula-Broca connections were localized to the dysgranular portion of the anterior insula; insula-LMC connections were found in the dysgranular insula more centrally and closer to its

posterior portion, and insula-Wernicke connections were distributed in the granular posterior insula. Insula-LMC and insula-Wernicke connections showed left-hemispheric lateralization. Larger-scale insular connectivity patterns were characterized by the representation of the strongest connections in the hippocampus, superior orbital, middle occipital, and middle frontal gyri. Conversely, SD patients showed abnormal overlap of the insular connection partitions and a larger representation of the insula-LMC connections, especially in the right hemisphere. The left-hemispheric distribution of insular connections seen in HV was diminished with a more bilateral representation of insular fiber tracts. Subsequently, the larger-scale insular connectivity was also different from that in HV and included SD-specific nodes in the sensorimotor, middle cingulate, inferior temporal, and inferior parietal cortices.
We demonstrated anatomical segregation of the large-scale insular network, specifically involving the cortical regions responsible for different aspects of speech processing and production. This organization appears to be altered and skewed towards motor overrepresentations in a neurological disorder affecting speech production. Such differences in insular (dis)organization may underlie distinct symptomatology due to the insular insult affecting its different divisions.

Disclosures: **G. Battistella:** None. **V. Kumar:** None. **K. Simonyan:** None.

Poster

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Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Title: Prosodic influence in face emotion perception: evidence from functional near-infrared spectroscopy

Authors: *K. M. BECKER, D. C. ROJAS;
Psychology, Colorado State Univ., Fort Collins, CO

Abstract: The ability to make inferences about the emotional state of another individual is facilitated by the seemingly automatic integration of concurrently presented vocal and facial information. Humans possess a special neural network dedicated to the recognition of facial expressions, which is distinct from brain regions devoted to face detection and prosody recognition. This study aims to examine the neural correlates of these responses by measuring changes in oxygenated hemoglobin (HbO) using functional near-infrared spectroscopy. Prosodic stimuli consisted of affective vocalizations of the sound “ah” produced in neutral, angry, and happy tones. Face stimuli were made by morphing the images of an actor making a happy and an

angry face to create a continuum of faces that varied from 100% happy to 100% angry. These stimuli were used to create six conditions, allowing for the presentation of bimodal (face and voice) and voice only in all three prosodies. An additional face only condition was added to further contrast bimodal and prosodic influences in emotion perception. One-hundred and five channels of fNIRS data were acquired using a NIRx Medical Systems NIRScout Extended system. Data were processed in the SPM_fNIRS software package. Bimodal happy stimuli exhibited greater concentrations of HbO bilaterally in the temporal-parietal junction (TPJ) region when compared to the bimodal neutral condition, whereas bimodal angry stimuli were shown to have decreased HbO concentrations when compared to the neutral stimuli. Additionally, across conditions, bimodal stimuli showed decreased HbO in the posterior superior temporal sulcus (pSTS) of the right hemisphere when contrasted with the voice only condition. When compared to the face only condition, the voice only conditions exhibited greater activation in the supramarginal gyrus bilaterally, greater HbO concentrations in the somatosensory cortex of the left hemisphere, and increased HbO concentrations in the supplementary motor cortex of the right hemisphere. These findings indicate that a constellation of brain areas contribute to emotion perception, with TPJ and pSTS areas playing a key role in multisensory integration of emotion perception.

Disclosures: K.M. Becker: None. **D.C. Rojas:** None.

Poster

721. Motor Systems: Oral Motor and Speech

Location: Halls B-H

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Program#/Poster#: 721.06/TT8

Topic: E.04. Voluntary Movements

Support: The NYSC Foundation

NIH Grant 1TL1 TR001447

Title: Characterization of a song locus in the right posterior superior temporal gyrus via electrical stimulation and focal cooling during awake neurosurgery

Authors: *K. KATLOWITZ[1], H. OYA[2], M. A. HOWARD[2], J. D. W. GREENLEE[2], M. A. LONG[1];
[1]New York Univ., New York, NY; [2]Dept. of Neurosurg., Univ. of Iowa, Iowa City, IA

Abstract: While the right hemisphere has been shown to be preferentially involved in music, the precise roles of the individual cortical loci that underlie this lateralization are still being

characterized. In a case study during an awake craniotomy, we found that electrical stimulation to a single locus in the right posterior superior temporal gyrus selectively interrupted singing but not speaking. We further characterized the role of this region with focal cooling during the production of simple sequences of either speech or singing. We found no noticeable defects in vocal quality or timing in either task. Unexpectedly, consistent pitch shifts were detected for spoken words but not singing. Identifiable changes in formants were detected in both vocal tasks. These results highlight the specialized processing of higher order vocal parameters in the right posterior superior temporal gyrus and suggest that a multi-modal approach is necessary for characterizing these factors.

Disclosures: K. Katlowitz: None. **H. Oya:** None. **M.A. Howard:** None. **J.D.W. Greenlee:** None. **M.A. Long:** None.

Poster

721. Motor Systems: Oral Motor and Speech

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CIHR Grant MOP-137001

Title: Pre-speech auditory modulation in a picture naming task

Authors: A. HANS[1], A. DALIRI[2], *L. MAX[1,3];
[1]Univ. Washington, Seattle, WA; [2]Boston Univ., Boston, MA; [3]Haskins Labs., New Haven, CT

Abstract: Sensorimotor integration for voluntary movements involves predictions that are used to optimize motor commands and to respond appropriately to sensory inputs. It is well documented, for example, that the central nervous system (CNS) modulates its response to self-generated vs. externally-generated afferent inputs. In the speech domain, cortical responses to self-generated vocalizations are reduced in comparison with responses to a played-back version of the same vocalizations. Current theoretical models suggest that such sensory modulation during movement execution occurs when the CNS detects a match between predicted and actual sensory consequences. Using EEG recordings and probe stimuli delivered prior to speech onset, our own lab has repeatedly demonstrated that motor-to-sensory signals already start to modulate auditory processing during the planning phase that preceeds movement execution. In our basic

paradigm, written words were presented on a screen, and we recorded long latency auditory evoked potentials (LLAEPs) in response to probe tones presented while the participant was planning to say the word out loud vs. a control condition with only silent reading. With a view to studying the phenomenon of pre-speech auditory modulation also in children, we have now developed a modified protocol that involves picture naming rather than word reading. In this new protocol, a probe tone is played after a picture is shown on a screen but before a green border appears around the picture. In 'speaking' blocks of trials, each picture is named when the green border appears, and in 'no-speaking' blocks of trials, each picture is viewed silently. LLAEPs are recorded to measure the auditory system's responses to the probe tones. In a first experiment, we validated the new paradigm with a group of adult participants to ensure that the results could be directly compared with those from our prior studies. Findings confirmed that pre-speech auditory modulation during the picture naming task is indeed comparable to that previously observed during our word reading task. In a second experiment, we have started investigating whether the duration of a data collection session can be reduced - without negative effects on the ability to detect pre-speech auditory modulation - by presenting more than one auditory stimulus per trial. Again we are initially testing adult participants to avoid confounding age effects and protocol effects. Overall findings from these experiments with the novel picture naming task confirm its sensitivity for detecting pre-speech auditory modulation, and, thus, support its application in future studies with children as participants.

Disclosures: **A. Hans:** None. **A. Daliri:** None. **L. Max:** None.

Poster

721. Motor Systems: Oral Motor and Speech

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Program#/Poster#: 721.08/TT10

Topic: E.04. Voluntary Movements

Support: Ohio University PI Start-Up Funds

Title: Multisensory perturbation effects on speech timing and sequencing

Authors: *F.-X. BRAJOT[1], J. LEE[2];
[1]Communication Sci. and Disorders, [2]Ohio Univ., Athens, OH

Abstract: The purpose of this pilot study was to evaluate the impact of vibration at the neck on healthy adults' responses to a temporal perturbation of auditory feedback in order to further delimit the integrated role of somatosensory and auditory feedback on the on-going coordination of speech gestures. Monolingual, native speakers of English read sentences in four

sensory feedback conditions: normal feedback, delayed auditory feedback, vibration at the neck, and delayed auditory feedback plus vibration. Vibrotactile stimulation resulted in a decrease in speaking rate, but speakers were otherwise fluent relative to the baseline condition. Delayed auditory feedback alone lead to a decrease in speaking rate, as well as an increase in overall number of speech errors, including sound, syllable and word-level repetitions. Combining vibration to the neck with delayed auditory feedback further decreased speaking rate and increased the number of speech errors, beyond what was observed for delayed auditory feedback alone. Results support previous research demonstrating phonatory/articulatory interactions and further establish a role for the perilaryngeal somatosensory system in the temporal coordination of speech.

Disclosures: **F. Brajot:** A. Employment/Salary (full or part-time): Ohio University. **J. Lee:** None.

Poster

721. Motor Systems: Oral Motor and Speech

Location: Halls B-H

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Program#/Poster#: 721.09/TT11

Topic: E.04. Voluntary Movements

Support: R21DC013915

Title: Orofacial somatosensory inputs enhance the change of speech perception induced by speech motor learning

Authors: ***H. OHASHI**[1], T. ITO[1,2,3];
[1]Haskins Labs. Inc, New Haven, CT; [2]Gipsa-lab, CNRS, Saint Martin D'heres Cedex, France; [3]Univ. Grenoble-Alpes, Grenoble, France

Abstract: Somatosensory input alters speech perception (Ito et al., 2009; Gick & Derrick 2009) even when listening to speech. One possible explanation is that during development, perceptual representations for speech are encoded from the integration of motor action and their auditory and somatosensory consequences. The resulting representations are sensorimotor in nature. As a result, somatosensory stimulation during speech perceptual discrimination is decoded as a change in motor action. To test this hypothesis, we focused on the change of speech perception induced by speech motor learning and examined whether a change in speech perception can be modified by additional somatosensory inputs associated with facial skin deformation. We tested native speakers of American English for the production and perception of /s/ and /sh/ consonants. For the speech training, subjects were asked to repeat the utterance ‘a shed’ under conditions of

altered auditory feedback. The produced consonant sound /sh/ was feedback to the subjects with modulation of the spectral centroid frequency (Shiller et al., 2009). The spectral centroid was increased resulting in acoustic signal properties closer to those of /s/. During adaptation, somatosensory stimulation associated with facial skin stretch was synchronized with the articulatory motion for the production of /sh/. The direction of skin stretch was opposite to the primary direction of skin deformation for the production of the target consonant, /sh/. Perceptual discrimination testing using an /s/-/sh/ acoustic continuum was carried out prior to and immediately after speech training. In a control group of participants, skin stretch was not presented during a training session. The degree of the speech motor adaptation was quantified as a change in spectral centroid frequency of the target consonant /sh/. Perceptual discrimination was evaluated as a change in the detection threshold for the /s/ and /sh/ consonants. Both groups showed significant adaptation to the altered auditory feedback and change in the perceptual boundary between /s/ and /sh/ consonants. Most notably, we found that the perceptual shift in the skin-stretched group was greater than that in the control group indicating additional somatosensory input during training enhanced the perceptual shift induced by speech motor learning. The result suggests that speech production is linked to the perceptual processing of speech through somatosensation during learning. Moreover, the data provide support for speech perception as a sensorimotor process.

Disclosures: H. Ohashi: None. **T. Ito:** None.

Poster

721. Motor Systems: Oral Motor and Speech

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Topic: E.04. Voluntary Movements

Title: Paralyzed recurrent laryngeal nerves demonstrate significant differences in morphometric parameters compared with non-paralyzed nerve

Authors: *M. B. CHRISTENSEN[1], A. HELLER[2], M. R. CHAO[1], K. A. HOWE[3], J. L. PIERCE[2], M. E. SMITH[4];
[1]Bioengineering, [2]Communication Sci. and Disorders, [3]Biol., [4]Otolaryngology/Head and Neck Surgery, Univ. of Utah, Salt Lake City, UT

Abstract: Vocal fold paralysis can be treated by surgically connecting the paralyzed recurrent laryngeal nerve (RLN) to a branch of the ansa cervicalis. This procedure has a good success record in restoring voice, although results vary from patient to patient. This may be due to a variety of factors that have not been well studied. One of these may be the degenerative state of

the paralyzed nerve, which can be investigated by histological studies. Morphometric measurements can be taken from these specimens which yield quantitative information about the nerve, including myelinated fiber counts, densities, packing, and g-ratio and fiber diameter distributions. This study describes the morphometric characteristics of paralyzed laryngeal nerve specimens and compares those to non-paralyzed nerves. RLN specimens from patients with unilateral RLN paralysis who underwent a laryngeal reinnervation procedure were processed using plastic embedding, sectioning, and staining techniques. High-power montage images were collected from each fascicle in the nerves and processed through custom LabVIEW-based programs to identify individual fibers. Morphometric parameters from each fiber were then collected using ImagePro Plus. Control data from RLN samples collected from laryngectomy specimens were also collected. Paralyzed nerves show varying degrees of degeneration. Overall, paralyzed nerves have significantly lower fiber counts, densities, and packing (percent of fascicle area occupied by fibers) than control nerves. Mean g-ratio values for paralyzed nerves were significantly higher than non-paralyzed nerves, indicating thinner myelination. Fiber diameter distributions were also significantly shifted towards smaller diameter fibers. It is unclear if this is due to a preferential loss of large diameter fibers, or a general loss of fibers followed by collateral sprouting during limited regeneration. Further analysis will compare the state of degeneration in the nerve to the amount of time between onset of paralysis and tissue collection. Morphometric data can also be compared with clinical data such as age, gender, cause of injury, laryngeal EMG, and voice outcome from reinnervation. This may provide insights into patient selection to improve the outcomes of laryngeal reinnervation for vocal fold paralysis.

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Poster

721. Motor Systems: Oral Motor and Speech

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Topic: E.04. Voluntary Movements

Support: Faculty of Dentistry Dental Research Institute Fund

Canadian Institute of Health

Title: Unilateral molar tooth extraction induces changes in the number and morphological features of astroglial cells within mouse orofacial motor cortex

Authors: T. WATASE[1], D. TANG[2], P. CHERKAS[2], D. K. LAM[2], S. BEGGS[3], M. SALTER[3], Z. SELTZER[2], B. J. SESSLE[2], *L. AVIVI-ARBER[4];
[1]Nihon Univ., Tokyo, Japan; [2]Univ. of Toronto, Toronto, ON, Canada; [3]Sick Kids Hosp., Toronto, ON, Canada; [4]Univ. of Toronto Dent., Toronto, ON, Canada

Abstract: **Background**: We have shown in rodents that orofacial injury, including tooth extraction, is associated with functional plasticity in the orofacial primary motor cortex (oM) and trigeminal medullary dorsal horn neurons, and that the latter changes are associated with changes in the number and volume of medullary astroglial cells. **Objective**: To determine if changes occur in the number and 3D morphological features of astroglia in the oM following extraction of right maxillary molar teeth in mice. **Methods**: Under Isoflurane general anaesthesia, young female BXA-24 mice had either extraction of the right maxillary molar teeth or sham operation, and naïve mice had no treatment (n=5/group). Mice were fixation-perfused on day 21 following extraction or sham operation. Coronal cryosections (40μm) of the oM region were immunolabeled with anti-GFAP (an astroglial marker). Whole slides were scanned with an Aperio Scanscope at 20x magnification. CaseViewer and Panoramic software were used to select regions of interests (ROI) within layers 1-2 (600 x 75 μm^2) and 5 (300 x 300 μm^2) within the left oM of 5 slices/mouse, and the numbers of astroglial cells were counted manually. In addition, 3D Z-stack images were collected using a spinning disk confocal microscope equipped with 63x/1.3 (water) objective lens, and Bitplane Imaris software was used to quantify the volume and morphological features of astroglial processes within ROIs in layers 1-2 (155 x 75 x 15 μm^3) and 5 (155 x 155 x 15 μm^3) of the left oM. Statistical analysis used ANOVA followed by *post-hoc* Bonferroni, $p<0.05$ was considered statistically significant. **Results**: In comparison with naïve and sham mice, tooth extraction was associated with a significantly increased number of astroglia in layers 1-2 and 5 of oM ($p<0.05$), as well as increased surface area and volume of astroglial processes ($p<0.01$). **Conclusions**: Together with our previous published data, these novel findings suggest that astroglial cells are involved in the mechanisms underlying oM changes following tooth loss, and that oM astroglia may represent a potential novel target for modulating orofacial sensorimotor functions following orofacial injury.

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Poster

721. Motor Systems: Oral Motor and Speech

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Topic: E.04. Voluntary Movements

Support: NIH Grant R01 NS026413

Yale GSAS

Title: Neural mechanisms underlying temporal organization in mouse courtship vocalizations

Authors: *G. A. CASTELLUCCI[1], E. W. ZAGHA[2], D. A. MCCORMICK[2];
[1]Neuroscience, Linguistics, [2]Neurosci., Yale Univ., New Haven, CT

Abstract: Vocalizations produced by a wide variety of organisms - including pinnipeds, songbirds, and humans - display a high degree of temporal regularity. We have recently demonstrated that the ultrasonic courtship vocalizations ("song") of male mice also exhibit a characteristic rhythmic structure composed of stereotyped alternations between two temporally distinct call ("syllable") types and three silent interval ("boundary") classes (Castellucci et al. 2016, *Sci. Rep.*, 23305). In the present study, we demonstrate that this rhythmic structure is an archetypal characteristic of wildtype (C57Bl/6J) song, and the temporal features of syllables as well as bouts of syllables are extremely stable across mice. We also observed distinct temporal signatures in syllables adjacent to boundaries and transitions between syllables types, indicating that this vocal rhythmic structure may be planned to some extent by the motor system. We have also begun examining motor coordination during vocalization production using freely moving plethysmography and vocal tract electromyography to better understand the articulatory strategies used in syllable and boundary production. For example, we have determined that different boundary types are articulated with distinct patterns of respiratory activity. Finally, despite the high degree of stability in the temporal organization of vocalizations across mice, we found that disruptions in *Foxp2* expression and lesions to the cerebellum result in temporal defects in song production. Interestingly, the speech of humans with mutations in the *FOXP2* gene or damage to the cerebellum also displays timing and prosodic irregularities, suggesting that the study of the murine vocalization production system may prove to be a valuable model in which to study the biological basis of temporal organization in speech and diseases that affect it.

Disclosures: G.A. Castellucci: None. **E.W. Zagha:** None. **D.A. McCormick:** None.

Poster

721. Motor Systems: Oral Motor and Speech

Location: Halls B-H

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Program#/Poster#: 721.13/UU1

Topic: C.03. Parkinson's Disease

Title: A model comparison guide of DNA double-strand breaks in the brain and tongue

Authors: *G. TORRES[1], J. R. LEHESTE[2];
[1]NYIT COM, Old Westbury, NY; [2]Biomed. Sci., NYIT-COM, Old Westbury, NY

Abstract: DNA damage occurs throughout the entire lifetime of neurons due in part to their high rates of oxygen metabolism, post-mitotic state and relatively long life span. Some damage may have an evolutionary function; however, the majority of the damage occurs spontaneously and can alter key cellular function contributing to pathology. DNA double-strand breaks are a type of molecular lesion in which the double helix structure is physically broken at specific genomic loci. While DNA double-strand breaks are widespread in developing and mature nervous system landscapes, deficiencies in repair of nuclear and mitochondrial DNA damage have been linked to several neurodegenerative disorders, including Parkinson's disease (PD). The objective of the present study is to use the animal model of PD, the PIT3X mouse, to address the issue of DNA double-strand breaks in the brain. In addition, we address the extent of DNA double-strand breaks in the tongue as structural and nuclear changes are widespread in PD, including cell pathways involved with energy sensing and metabolic control. It should be noted that the tongue is heavily involved in the physiology of perception, with emphasis on gustation and chemesthesis. In addition, electrical signals from the tongue yield sustained activation within the medial orbito-frontal cortex and hippocampus, indicating a reward response. In this context, PD patients often present with deficits in olfactory and gustatory sensing along with core motor features of the disease, including tremor, bradykinesia and muscle rigidity. These findings suggest that the ability of the tongue to probe calorie content and taste signaling may be diminished in PD as a result of excessive DNA double-strand breaks. Indeed, our preliminary results indicate that the hippocampus and tongue of PIT3X mice appear to differ in their relative load of DNA double-strand breaks from that of healthy control C57BL/6J mice. Further insight on the relative contribution and potential synergies of the brain and tongue to PD pathology is needed to refine applicable programs of therapy for this particular neurodegenerative disease.

Disclosures: G. Torres: None. **J.R. Leheste:** None.

Poster

722. Posture and Gait: Higher Order Control

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Topic: E.06. Posture and Gait

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Eric P. and Evelyn E. Newman Fund

Title: Ankle impedance modulation evoked by imposed inversion/eversion stiffness during locomotion

Authors: C. ARNET[1], J. OCHOA[1], D. STERNAD[2], *N. HOGAN[1];
[1]MIT, Cambridge, MA; [2]Northeastern Univ., Boston, MA

Abstract: As a distal joint, the ankle is responsible for mechanical interaction between the human body and the ground. Previous research showed that humans modulate ankle mechanical impedance during walking: for most of the swing phase, ankle impedance is low, but it increases towards the end of the swing phase prior to heel-strike, when its action resembles a 'shock absorber'. However, previous research also showed that the human ankle is especially weak and compliant in inversion, consistent with the prevalent direction of ankle sprains. In this study we examined the effect of externally-applied inversion/eversion (IE) stiffness, both stabilizing (positive) and de-stabilizing (negative), with applications to the treatment of chronic ankle instability. The experiments studied healthy subjects walking on both a treadmill and overground while a wearable ankle robot imposed a programmable IE stiffness. During locomotion, the robot recorded the knee angle profile and ankle excursions in the IE and DP (dorsi/plantarflexion) directions. Muscle activity was recorded from Tibialis Anterior, Peroneus Longus, Gastrocnemius, and Soleus using surface electromyography (EMG). Pressure sensors under the heel and toes identified the heel-strike and toe-off phases of the gait cycle. Trials were divided into four five-minute epochs, administering neutral, positive, and negative stiffness in the IE direction only; no external stiffness was applied in the DP direction. Each epoch allowed for sufficient time to assess immediate response and possible adaptation to the imposed stiffness. First, zero stiffness was applied to establish a baseline. Subsequently, positive stiffness (40 Nm/rad)—analogous to walking with an IE brace—was applied. IE stiffness was then returned to zero to assess the persistence of any adaptation. Next, IE stiffness was decreased to a negative value (-10 Nm/rad)—analogous to walking on a thin pipe. Lastly, the imposed stiffness returned to zero to assess the persistence of any adaptation induced by the negative stiffness. As expected, imposing positive IE stiffness evoked a decreased range of IE motion but did not affect range of DP motion. Imposing negative IE stiffness evoked an increased range of IE motion. Remarkably, it also evoked a reduced range of DP motion, consistent with increased neuromechanical stiffness in both DP and IE. The latter may be due to restoring the stability margin reduced by the negative external stiffness, the former due to coupling between degrees of freedom. These results suggest that maintaining adequate ankle stiffness is an important aspect of human locomotion and may have implications for robot-aided locomotion therapy.

Disclosures: **C. Arnet:** None. **J. Ochoa:** None. **D. Sternad:** None. **N. Hogan:** None.

Poster

722. Posture and Gait: Higher Order Control

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Program#/Poster#: 722.02/UU3

Topic: E.06. Posture and Gait

Title: The effect of optic flow direction on orthogonality of balance control during walking

Authors: *J. NIELSEN;
Biomechanics, Univ. of Nebraska at Omaha, Omaha, NE

Abstract: Background: A healthy sensorimotor system maintains spatial parameters (step-length and step-width) within a range of variability during walking. It has been seen that the influence on spatial variability is direction-dependent such as when walking in the anterior-posterior plane (forwards), step-length is consistent while the orthogonal, step-width, is significantly more variable. Consequently, when walking in the sagittal plane (sideways) there is a reversal of this variability. This has led to the inference that orthogonality of balance control, indicated by the characteristic orthogonality of movement variability during walking, is governed by the direction of progression. However, it is not clear how visual flow information affects this orthogonality. **Methods:** Participants walked on a treadmill while wearing a virtual reality (VR) headset. Once a preferred walking speed was established (PWS) for both anterior-posterior (AP) and medial-lateral (ML) walking, familiarization trials with the headset were performed in the AP and ML directions. Subsequently, participants randomly performed 8 trials - 4 VR conditions in each of the two directions. The VR conditions consisted of walking in a VR corridor that provided: AP optic flow (OF), ML OF, diagonal OF, and an Oscillating OF. Coefficient of variation was calculated for step-length and step-width. **Results:** The effect of walking direction led to a ten-fold increase in variability in the orthogonal direction. The effect of VR was the following: In comparison to the congruent OF conditions (i.e., OF in AP for AP walking and ML for ML walking), walking trials with the orthogonal OF showed a 6.6% increase, the diagonal OF, a 0.6% increase, and the oscillating OF, an 8.6% increase in COV. **Discussion:** During trials with conflicting locomotor and OF directions there was an increase in variability between steps. This shows the importance of reliable visual perception of self-motion for maintaining balance control during dynamic tasks such as walking.

Disclosures: **J. Nielsen:** None.

Poster

722. Posture and Gait: Higher Order Control

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 722.03/UU4

Topic: E.06. Posture and Gait

Support: Imperial College London

Title: Effects of hand posture on corticospinal excitability to trunk muscles

Authors: *J. K. WONG, S. CHIDAMBARAM, S.-Y. CHIOU, P. H. STRUTTON;
Dept. of Surgery and Cancer, Fac. of Med., Imperial Col. London, London, United Kingdom

Abstract: Trunk muscles are critical for the successful performance of many daily tasks. They maintain postural equilibrium in response to external and internal perturbations, such as upper limb movements. We have shown that corticospinal excitability to the trunk muscles is increased during dynamic shoulder flexion indicating interactions between the neural pathways controlling upper limb and trunk muscles in a postural task. Further, we have shown that increases in trunk corticospinal excitability also occur during static contractions of certain upper limb muscles. Whether cortical input to trunk muscles can also be facilitated during functional hand movements, in which the postural disturbance is less, is unclear and was the purpose of this study.

Twenty healthy subjects (mean [SD] age 22.1 [1.1] years; 12 male, 8 female) performed static hand tasks with varying grips (power, precision) and postures (neutral, pronated, supinated) with the dominant hand. Transcranial magnetic stimulation at an intensity of 120% of active motor threshold was delivered to the primary motor cortex targeting the hotspot for the contralateral erector spinae (ES) during the tasks. The resulting motor evoked potentials (MEPs) in ES were recorded using electromyography (EMG). ES MEP amplitudes during different tasks were normalised to a control task to allow inter-subject comparisons. Pre-stimulus EMG recorded from the ES was matched across all tasks to ensure consistent levels of motoneuron excitability. The normalised ES MEP amplitudes were higher for the neutral hand posture (112.61±9.06%), than the supinated posture (97.77±9.23%) during a power grip ($p<0.01$). There were no differences in MEP amplitudes between postures for the precision grip. Further, there were no differences for a given posture between grip types ($p>0.05$). These findings suggest that crossed facilitation exists between hand and trunk muscles and grasping with different hand postures may have distinct effects on trunk corticospinal excitability. The clinical implications of the current findings include rehabilitation, where improvements in trunk control in people with deficits in trunk control such as stroke and spinal cord injury, using repetition of hand movements, might be possible.

Disclosures: **J.K. Wong:** None. **S. Chidambaram:** None. **S. Chiou:** None. **P.H. Strutton:** None.

Poster

722. Posture and Gait: Higher Order Control

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Topic: E.06. Posture and Gait

Support: NIH NICHDK01 HD079584 (Kesar)

NIHK12 HD055931 (Borich)

Title: Effect of co-contraction on cortical excitability of lower limb musculature

Authors: ***T. M. KESAR**[1], M. R. BORICH[1], S. EICHOLTZ[2], S. L. WOLF[1];
[1]Physical Therapy, 1441 Clifton Rd NE, Room 205, Atlanta, GA; [2]Emory Univ., Atlanta, GA

Abstract: INTRODUCTION: Transcranial magnetic stimulation (TMS) has been extensively used to evaluate corticospinal circuitry, but the majority of studies have investigated upper extremity musculature. There is a paucity of investigations on lower limb muscles, and on how methodological aspects such as agonist or antagonist activation influence TMS-evoked responses. Here, our objective was to evaluate the effect of agonist-antagonist co-contraction on TMS-evoked motor evoked potentials (MEPs) recorded from the tibialis anterior (TA) muscle.
METHODS: 13 young, neurologically-unimpaired adults (3 male, 10 female, ages 23-35 years) participated in this study. TMS pulses were delivered at 120% resting motor threshold over the TA hotspot, and TMS-evoked MEPs were recorded from the TA muscle using surface electromyography (EMG). Three conditions were tested: TA and soleus at rest (Rest), TA activated at 10% maximum EMG activation (Agonist-on), and both TA and soleus activated at 10% of maximal activation (Co-contraction). Paired t-tests were performed to compare TA MEPs as well as pre-stimulus background EMG amplitude during the rest and co-contraction conditions with the Agonist-on condition.
RESULTS: Comparison of background EMG amplitudes confirmed that the TA background activation was greater during Agonist-on and Co-contraction versus Rest. There were no differences in TA background EMG during the Agonist-on versus Co-contraction conditions. Compared to the Rest and the Agonist-on conditions, TA MEP amplitudes were significantly greater during the TA-soleus Co-contraction condition ($p<0.05$). Ongoing analysis is exploring whether cortical excitability of proximal lower limb muscles (e.g. quadriceps) are influenced by co-contraction at the ankle joint.

DISCUSSION: Our results suggest that during the measurement of TMS-evoked MEPs, agonist-antagonist co-contraction may have potential as a testing condition that provides enhanced cortical excitability and more controlled muscle activation states. Greater cortical excitability observed during the Co-activation condition may be caused by several factors. Co-activation may require greater cognitive effort and cortical activation. Additionally, previous studies suggest that TA and soleus may have overlapping cortical representations. Furthermore, due to the location of the lower extremity M1 deep within the inter-hemispheric fissure, both the TA and soleus corticomotor-neurons may be activated by suprathreshold TMS. Future studies will determine to what extent aging, neurologic impairment, and gait training influence the effects of muscle activation on cortical excitability.

Disclosures: **T.M. Kesar:** None. **M.R. Borich:** None. **S. Eicholtz:** None. **S.L. Wolf:** None.

Poster

722. Posture and Gait: Higher Order Control

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Program#/Poster#: 722.05/UU6

Topic: E.06. Posture and Gait

Title: Effect of concurrent cognitive task on postural stability and postural task-related cortical activation.

Authors: *H. OTOMUNE[1,2], M. MIHARA[1,3], Y. KAJIYAMA[2], Y. GON[2], H. FUJIMOTO[2], K. KONAKA[2], T. KAWANO[1], M. NAGASAKO[1], T. YOSHIOKA[1], M. HATAKENAKA[1], H. YAGURA[1], I. MIYAI[1], H. MOCHIZUKI[2];
[1]Neurorehabilitaion Res. Inst., Morinomiya Hosp., Osaka-Shi, Japan; [2]Osaka Univ. Grad. Sch. of Med., Osaka, Japan; [3]Div. of Clin. Neuroengineering, Osaka Univ., Suita, Japan

Abstract: Objective:Clinical observations that supratentorial lesions often impair postural balance suggested an important role of the cerebral cortex in human bipedal postural control. Accordingly, concurrent cognitive task (dual-task) often leads to impaired performance of postural or gait task in elderly people. However, several studies have shown that postural stability is improved under dual-task condition in younger people. These paradoxical findings raise a question about the cortical role for the human postural control. To investigate the cortical mechanisms for this paradoxical finding, we directly assess the postural task related cortical activation using functional Near infrared spectroscopy (fNIRS) system. Methods:We recruited 15 healthy young subjects. For the postural task, we applied brisk forward and backward translations of a platform as a perturbation. Fourteen perturbations were delivered with randomized intervals of 10-18 s. For the cognitive task, we asked the subjects to perform flanker

task in the upright position. For the dual task, they performed flanker task between perturbations. As a measure of balance ability, the cumulative displacement of the position of the center of pressure (COP) during three seconds around the perturbation. To detect task-related cortical activation, statistical analysis using general-linear model were performed with Oxy-Hb based signal. Results:Multi-subject's analysis of Oxy-Hb signals showed that prefrontal cortical areas in both hemispheres as well as the left supplementaly motor area significantly activated in simple postural task than dual-task . On the other hand, there was no area more activated in dual-task than simple postural task. In behavioral analysis, mean COP sway by perturbations was smaller in dual-task than simple postural task. Conclusions:Our findings suggested the better postural control in dual-task condition would associate with the smaller cortical activation, But there would be several possible interpretation including less cortical demand lead to more automatic, reflexive postural control resulting in better postural control, or predictive recruitment of the cortical resource for upcoming perturbation would reduce task-related cortical activation in dual-task condition.

Disclosures: **H. Otomune:** None. **M. Mihara:** None. **Y. Kajiyama:** None. **Y. Gon:** None. **H. Fujimoto:** None. **K. Konaka:** None. **T. Kawano:** None. **M. Nagasako:** None. **T. Yoshioka:** None. **M. Hatakenaka:** None. **H. Yagura:** None. **I. Miyai:** None. **H. Mochizuki:** None.

Poster

722. Posture and Gait: Higher Order Control

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Program#/Poster#: 722.06/UU7

Topic: E.06. Posture and Gait

Support: Imperial College London

Title: Effects of dynamic arm cycling on crossed-corticospinal facilitation to trunk muscles

Authors: *D. CHRISTMAS, S. DAVE, S.-Y. CHIOU, P. H. STRUTTON;
Imperial Col. London, London, United Kingdom

Abstract: Voluntary use of the upper limb muscles in a static isometric contraction has previously been shown to increase corticospinal excitability to the trunk muscles, known as crossed-corticospinal facilitation. These interactions may underlie the postural adjustments associated with upper limb movements. Evidence exists that an alternating dynamic task significantly increases the corticospinal excitability of upper limb muscles over an EMG matched static equivalent. Whether an alternating dynamic task produces similar increases in crossed-corticospinal facilitation in muscles of the trunk has not been studied. The aim of this

study was to determine whether the corticospinal excitability of the trunk muscles during a continuous unimanual cycling task differs to the excitability seen in a static upper limb task. 20 healthy subjects (mean [SD] age 23.6 [5.8] years) performed a dynamic task (forward arm cycling at 60 revolutions per minute), a static task (exerting force on the locked pedal) and a control task (hands resting on the thighs). EMG activity was recorded from the contralateral erector spinae (ES) at the T12 vertebral level and rectus abdominis (RA) and from biceps brachii (BB) and the triceps brachii (TB) of the cycling arm. Transcranial magnetic stimulation was applied to the hotspot for ES over the primary motor cortex at the pedal position corresponding to maximal BB EMG (6 o'clock position) and maximal TB EMG (12 o'clock position). Prestimulus EMG in ES was matched within the 3 tasks for the different pedal positions. The study demonstrated that the size of MEPs for ES in dynamic and static tasks were significantly larger than the control in the 6 o'clock pedal position, but there were no significant differences in ES MEPs in the 12 o'clock pedal position. This is in-keeping with a recent study suggesting that crossed corticospinal facilitation between the upper limb and the trunk is increased in a static isometric BB contraction but not in a static TB contraction. Our results suggest that for a task with minimal postural requirement, the corticospinal excitability of the trunk muscles is independent of contraction type of the upper limb muscle. This could have implications for development of rehabilitation programmes in subjects with impaired trunk control, such as spinal cord injury or low back pain.

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Poster

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Topic: E.06. Posture and Gait

Support: CIHR

Title: Definition of cerebral networks involved in straight walking and steering of gait in normal young human subjects using ^{18}F-FDG PET imaging

Authors: *J.-P. SOUCY[1,2], F. STARRS[3], C. PAQUETTE[4];
[1]Montreal Neurolog. Inst., Montreal, QC, Canada; [2]PERFORM Ctr., Concordia Univ., Montreal, QC, Canada; [3]Kinesiology and Physical Educ., [4]McGill Univ., Montreal, QC, Canada

Abstract: Introduction: Steering of gait is an essential element of goal-directed locomotion involving a high degree of sensorimotor integration. Gait steering and directional changes while

walking are complex tasks which place a significant burden on the CNS, especially as individuals age and are affected by a variety of neurological diseases. Current concepts on control of ambulation propose that steering of gait and straight walking are supervised by distinct neuronal networks. fMRI studies in subjects imagining they are walking found that volitional goals (steering) originate from SMA which then modulates more automatic brain stem structures controlling straight walking via basal ganglia loops. Of course, such protocols do not involve actual walking and cannot account for sensory-motor integration. Therefore, the **aim** of this study is to identify the neuronal networks involved in the control of gait (straight walking versus upright standing) and steering of gait (steering of gait versus straight walking) in young healthy subjects using ^{18}F-fluorodeoxy-glucose (^{18}F-FDG) Positron Emission Tomography (PET). It is **hypothesized** that superior parietal and sensorimotor regions will be activated during steering of gait and that occipital areas and supraspinal structures will be activated during straight walking. **Methodology**: ^{18}F-FDG-PET was used to assess rCMGIc in 7 healthy subjects (mean age= 25). On testing days, subjects performed 1 of 3 motor tasks for 40 minutes immediately after tracer injection (approx. 150 MBq of ^{18}F-FDG): 1) steering of gait, 2) straight walking and 3) upright standing. Image acquisition started ~15 minutes after task completion. Straight walking images were subtracted from steering of gait images and upright condition images were subtracted from straight walking images. Regions showing significant increases or decreases in glucose metabolism were thresholded at $p < .05$, and corrected for multiple comparisons. **Results & Conclusions**: During steering of gait, our subjects showed consistent activation of the intraparietal sulcus, sensorimotor cortex and cerebellar vermis. The straight walking network for its part showed recruitment of the visual areas of the occipital lobe, sensorimotor cortex and the cerebellar vermis.
^{18}F-FDG-PET allowed for measurement of whole-brain activations during complex locomotor tasks and showed consistent steering of gait and straight walking neural networks. Information on the integration of sensory information with motor controls in controls is crucial for developing rehabilitation therapies for pathological populations and understanding the compensatory mechanisms of normal aging.

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Poster

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Centre Hospitalo-Universitaire de Poitiers (MSA/20098A00812855)

Title: Activity of the subthalamic and pedonculopontine nuclei during initiation of gait: an electrophysiological approach in humans.

Authors: *A. COLLOMB-CLERC[1,2,3,4], C. KARACHI[1,2,3], A. DEMAIN[1,3], X. DREVELLE[1,3], A. VAN HAMME[1,3,4], J.-E. LE DOUGET[1,3], P. LAVIRON[1,3,5,4], S. FERNANDEZ VIDAL[1,3,5,6], L. MALLET[1,2,3], B. LAU[1,3], M.-L. WELTER[1,2,3,4];
[1]Inst. Du Cerveau Et De La Moelle Epinière, Paris, France; [2]Assitance publique Hopitaux Paris, Groupe Hostipatier Pitié Salpetrière, Paris, France; [3]Sorbonne Universités, UPMC Univ. Paris 6, INSERM UMR 1127, CNRS UMR 7225, Paris, France; [4]Plateforme PANAM, ICM, Paris, France; [5]CENIR, Paris, France; [6]Plateforme STIM, ICM, Paris, France

Abstract: Humans share locomotion with all vertebrates, a capacity relying on a phylogenetically conversed neuronal network able to autonomously organize the locomotor pattern in relationship with internal and external constraints. It involves the central pattern generators at the spinal level, the mesencephalic locomotor region (MLR), that includes the pedunculopontine (PPN) and the cuneiform nuclei, at the brainstem level, the basal ganglia, the cerebellum and various, mainly fronto-parietal, cortical areas. In cats, electrical or chemical modulation of the neuronal activity of the MLR, the subthalamic nucleus area and the cerebellum provokes locomotor activity and postural tone changes. In humans, imaginary of gait triggered gait-specific activity in the PPN neurons, but no or few changes in the subthalamic nucleus (STN). To further decipher the role of the PPN and the STN in the human locomotion and postural control, we recorded the neuronal activity of these structures during real bipedal gait in patients with deep brain stimulation (DBS) electrodes. We recorded local field potentials (LFP) during gait initiation in 26 patients with Parkinson's disease (PD) implanted bilaterally for STN (n=22) or PPN (n=4) DBS, and 2 patients with obsessive compulsive disorders (OCD) implanted bilaterally for STN-DBS. Gait initiation, that comprises a preparatory postural phase and an execution phase, was quantified by coupling kinetic, kinematic and electromyographic acquisitions. In both PD and OCD patients, we observed STN neuronal activity modulations during the gait initiation with i) a synchronization in the theta-alpha band during the preparatory phase, ii) a desynchronization in the beta band that started at the preparatory phase , iii) an increased theta-band and gamma synchronization for the first and following steps preparations. In PD patients, the gait initiation process differently affected PPN neuronal activity with an increased synchronization in the alpha and gamma bands that started at the time of the first step execution, and the following steps, with no significant changes during the preparatory phase. These results may suggest that, in human, the STN is mainly involved in the preparatory phases and the PPN in the rhythmic stepping activity during locomotion.

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Poster

722. Posture and Gait: Higher Order Control

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Japan Society for the Promotion of Science KAKENHI Grant 25870131

Title: Neural correlates of dual-task walking: influence of priority on attentional demands

Authors: S. SANGANI[1], T. KURAYAMA[2], *J. FUNG[3,1];
[1]Feil/Oberfeld/CRIR Res. Ctr., Jewish Rehabil. Hosp., Laval, QC, Canada; [2]Physical Therapy, Uekusa Gakuen Univ., Chiba, Japan; [3]McGill Univ., Montreal, QC, Canada

Abstract: Walking while simultaneously performing another task requires divided attention, eg. holding a cup without spilling. Stability control during gait alone requires attention, which may be compromised as the cognitive demand increases. Our primary goal was to investigate neural correlates associated with increased attentional demands when holding a hot coffee/tea cup versus a cup of room-temperature water while walking using functional near-infrared spectroscopy (fNIRS). Healthy young adults (n=11) and a stroke participant walked on a 3m long force-sensing treadmill (CMill, Motek-Forcelink). Cortical activation was acquired with a NIRScout system (NIRx) using a custom-built cap covering the frontal cortex. The protocol included repeated block trials consisting of four alternating blocks of standing (20s) and walking (25s) at a comfortable speed determined prior to the experiment. Five walking trials were performed, each consisting of four randomized conditions including holding a Styrofoam cup that was empty or filled with water, jelly or hot liquid. Participants held the cup in the dominant or non-paretic hand. The cortical hemodynamic response was quantified by concentration changes of oxygenated hemoglobin (oxyHb) in the frontal cortex. Cortical response maps were determined based on the general linear model using SPM (nirsLAB) by dividing the walking trial in three different time segments, acceleration, steady-state walking and deceleration. In healthy controls, walking while holding a hot beverage was associated with an increased activation of the dorsolateral prefrontal cortex (DLPFC) only during the acceleration and deceleration phase. In

addition, the comparison of walking with a liquid medium (water/coffee/tea) vs a semi-liquid medium (jello) resulted in activation of SMA and DLPFC during acceleration and steady-state walking phase. On the other hand, comparison of walking with water vs jello in stroke participant results in cortical activation of lesioned as well as contralesional hemispheres. During acceleration, DLPFC of the lesioned hemisphere showed increased activation while steady-state walking was associated with increased activation of the contralesional DLPFC. The deceleration phase showed bilateral activation of the prefrontal cortex. The results of the study suggest that walking with a hot beverage increases priority on attentional demands which are associated with activation of the prefrontal cortex during different phases of dual-task locomotion.

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Poster

722. Posture and Gait: Higher Order Control

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Topic: E.06. Posture and Gait

Support: Natural Science and Engineering Research Council of Canada

Title: EEG brain functional connectivity during static and dynamic reactive balance control

Authors: *J. PAROKARAN VARGHESE[1], W. E. MCILROY[2];
[2]Kinesiology, [1]Univ. of Waterloo, Waterloo, ON, Canada

Abstract: Human bipedal balance control is importantly dependent on rapid reactions to internal or external perturbations to stability. It is now believed that a distributed network is involved in such reactive balance control with a potential role for the cerebral cortex. Our potential understanding of the role of cortical regions during balance control will benefit from identification of both functional segregation and neural integration/connectivity. Previous EEG studies have shown that a whole body balance perturbation results in a negative potential (N1) that peaks around 100 ms after the perturbation onset and is widely distributed across fronto-central-parietal areas. In addition, an evoked N1 potential also exists during automatic balance reactions that occur continuously when one is standing still and is widely distributed across fronto-central-parietal areas. The widespread distribution of N1 during static and dynamic reactive balance control leads to speculation that an integrated activity of different neuronal assemblies might generate N1 rather than a single dipole. Hence, this study aims to explore the EEG functional connectivity linked to the N1 by examining the event-related phase coherence between pairs of 30 EEG electrodes recorded during reactions to external balance perturbations

and internal perturbations during standing still. Twelve young healthy adults performed 30 sec of quiet standing (Tandem Romberg stance) on a force plate. Another twelve young healthy adults performed feet-in-place balance reactions to temporally unpredictable whole body perturbations evoked by releasing the cable of a lean and release system. The grand average connectivity matrix revealed that a robust functional connectivity exists between fronto-central-parietal areas in theta (4-6 Hz), alpha (8-12 Hz), and beta (14-30 Hz) frequency bands during perturbation-evoked N1 and natural instability-evoked N1. These results are compatible with the idea that integrated activity of different neuronal assemblies segregated in frontal, central, and parietal cortical areas might be involved in generating complex reactive balance control strategies.

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Poster

722. Posture and Gait: Higher Order Control

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Topic: E.06. Posture and Gait

Support: German Federal Ministry of Education and Research (BMBF) (01EL1522I)

Title: Proprioception under dual task conditions in older adults. Does physical activity matter?

Authors: I. BRAGINA, *C. VOELCKER-REHAGE;
Inst. of Human Movement Sci. and Hlth., Technische Univ. Chemnitz, Chemnitz, Germany

Abstract: Aging is associated with declines in sensorimotor and cognitive functioning. Further, older adults often recruit additional cognitive resources to perform a sensorimotor task and/or to reach youth-like motor performance levels. This becomes especially apparent in situations when a person has to perform more than one task simultaneously (so-called dual- (DT) or multi-task situations). In this line, proprioceptive acuity, especially in lower limbs, has been shown to decline with increasing age and to require more cognitive resources. Proprioceptive information from the mechanoreceptors is necessary for the performance of successful movements. Changes in proprioceptive acuity may contribute to an enhanced risk of falling and lead to restrictions in everyday life.
Lifestyle factors, like regular physical activity are proven to benefit motor and cognitive performance in older adults. Also lower limb proprioceptive acuity seems to be higher in persons who are physically active (Ribeiro and Oliveira, 2010). Whether this finding applies to older adults under DT conditions as well is unclear.
We investigate proprioceptive acuity under single-task (ST) and DT conditions in a sample of 50

older adults between 65 and 75 years of age. Further, we analyze whether baseline activity level has an influence on proprioceptive acuity. Physical activity is measured with an adapted version of the IPAQ (international physical activity questionnaire). Proprioceptive acuity is assessed by an ankle position-matching task under ST and DT conditions. Under DT conditions, the matching task is performed simultaneously with a Random Number Generation Task (Albinet et al., 2006) in order to determine the attentional costs of proprioceptive acuity.
We expect that persons who are regularly physically active, reach better performance in the proprioceptive and cognitive tasks under ST conditions and further exhibit less DT costs during the motor and cognitive tasks under DT conditions. The early recognition of declines in proprioception may contribute to prevent falls in the future. Further, insights into the relationship between physical activity and processing of proprioceptive acuity may provide important insights for exercise recommendations for older adults.

Disclosures: I. Bragina: None. **C. Voelcker-Rehage:** None.

Poster

722. Posture and Gait: Higher Order Control

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Program#/Poster#: 722.12/UU13

Topic: E.06. Posture and Gait

Title: The role of stride frequency for transition from walk to run in humans

Authors: *E. A. HANSEN, A. M. NIELSEN, L. A. R. KRISTENSEN, M. VOIGT, P. MADELEINE;
Ctr. For Sensory-Motor Interaction (SMI), Aalborg Univ., Aalborg, Denmark

Abstract: A better understanding of the control of bipedal locomotion can contribute to the development of programs for enhancement of human function and performance. Researchers within e.g. neuroscience, physiology, and psychology have investigated an intriguing long-standing question: Why do humans spontaneously shift from walk to run, at a certain point during gradually increasing velocity? (1, 2). To investigate this, we recruited 26 healthy individuals (19 men and 7 women of age, height, and body mass of 26.3±5.4 years, 1.78±0.08 m, and 75.2±10 kg, respectively) for treadmill walking and running. We showed that calculated stride frequency at walk-to-run transition (70.6±3.2 strides per min) agreed with transition stride frequency (70.8±3.1 strides per min) predicted from stride frequencies occurring during behaviourally natural conditions of walking and running at freely chosen velocities and stride frequencies. The two stride frequencies used for prediction were considered attractors. And the prediction was that gait is shifted to running at the point where the walking stride frequency

starts to get closer to the running attractor than it is to the walking attractor. Agreement was based on Bland & Altman's statistics for assessing agreement between two methods (3). Previous research has focussed on transition velocity (1, 2) and optimisation theories based on minimisation of e.g. rate of energy turnover (4) or biomechanical loadings of the legs (5). The previously reported coincidence between energetically optimal and freely chosen stride frequencies in locomotion could reflect evolutionary development rather than deliberate motor control prioritising minimisation of energy turnover. We propose the central phenomenon of walk-to-run transition to be attributed to the following. Based on a dynamical systems approach, attractors in form of stride frequencies during behaviourally natural gait conditions (here, walking and running at freely chosen velocities and stride frequencies) occur. The attractors possibly reflect outputs from a non-hierarchical tripartite system consisting of spinal central pattern generators, their supraspinal input, and their sensory feedback. During walking at increasing velocity, gait is shifted to running at the point where the walking stride frequency starts to get closer to the running attractor than it is to the walking attractor. References: 1) Kram R *et al*. 1997 *J Exp Biol* **200**, 821-826. 2) Diedrich FJ & Warren WH 1995 *J Exp Psychol Hum Percept Perform* **21**, 183-202. 3) Bland JM & Altman DG 1986 *Lancet* **1**, 307-310. 4) Alexander R 2002 *Am J Hum Biol* **14**, 641-648. 5) Ranisavljev I *et al.* 2014 *Hum Mov Sci* **38**, 47-57.

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Poster

722. Posture and Gait: Higher Order Control

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Program#/Poster#: 722.13/UU14

Topic: E.06. Posture and Gait

Title: Adjacent motor cortical areas have distinct brain functional connectivity

Authors: *A. M. ALBISHI[1], J. SMITH[2], B. FISHER[2], J. KUTCH[2];
[1]USC, Los Angeles, CA; [2]USC, LOS ANGELOS, CA

Abstract: Background: Postural control studies suggest that primary motor area (M1) is responsible for volitional movement execution while supplementary motor area (SMA) plays a role in postural preparation. Support for the role of SMA in postural control would be provided by determining functional connectivity and establishing greater connection of SMA to critical postural control areas compared to M1. Both M1 and SMA have direct projections to the spinal cord, thus muscle representational maps can be obtained using transcranial magnetic stimulation (TMS). However, motor representational maps do not identify what brain areas are connected to

M1 and SMA. Resting-state functional magnetic resonance imaging (rs-fMRI) can be used to identify muscle-specific neural circuitries.This study will compare whole-brain functional connectivity (FC) of SMA and M1 representational areas of external oblique (EO) to gain insight into the differential function of SMA and M1 in the control of this muscle. **Purpose:** Determine the location of EO cortical representation and explore resting state (rs) FC in SMA and M1 among healthy adults. **Methods:** 13 adults participated. TMS mapping of M1 and SMA was conducted. MEP amplitudes for EO determined the Center of Gravity (CoG) in both M1 and SMA. The MNI coordinates of EO CoG in SMA and M1 were used to explore FC of these areas utilizing rs-fMRI. **Results:** MEPs were elicited consistently in M1 and SMA. MNI coordinates for EO CoG were determined for M1 and SMA. FC analysis demonstrate that anterior cingulate, basal ganglia and cerebellum are more connected to SMA; Prefrontal, precuneus, and parietal cortex are more connected to M1. **Conclusion:** While EO is represented in both SMA and M1, these representations are not functionally equivalent in their interaction with the rest of the brain. Therefore, SMA and M1 may play distinct roles in the control of this postural muscle. Greater connectivity of SMA to basal ganglia and cerebellum compared to M1 can support the distinct role of SMA in postural control.

Disclosures: **A.M. Albishi:** None. **J. Smith:** None. **B. Fisher:** None. **J. Kutch:** None.

Poster

722. Posture and Gait: Higher Order Control

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 722.14/VV1

Topic: E.06. Posture and Gait

Support: LASPAU/CAPES BEX 13722-13-1

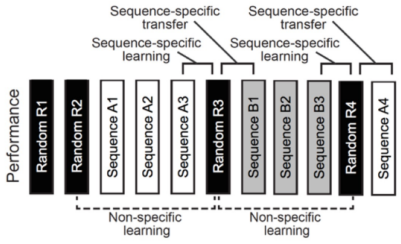
Title: Unilateral learning in a bilateral walking task

Authors: *G. BORIN, J. T. CHOI;
Kinesiology, Univ. of Massachusetts, Amherst, MA

Abstract: Complex motor skills, such as those involving sequencing, must be learned through repeated practice. In piano training, it is common to practice the left and right finger sequence separately before playing with both hands. Footwork sequence (e.g., for dancing) is usually practiced with both legs instead. Here we tested whether a learned bilateral step length sequences could be decomposed into individual leg components. If the sequence components were stored separately (unilaterally), then the two could be flexibly combined to produce new (untrained) bilateral walking patterns. In addition, we hypothesize that inter-limb transfer will occur if

learned sequences were stored as high-level task variables that could be implemented with either leg. **Methods:** Walking was challenged by presenting visual stepping targets displayed on a screen in the front of the treadmill. Subjects had to take different step lengths (i.e., short, medium or long) to hit the targets. Training consisted of both random blocks (with no repeating sequence) and sequence blocks (Figure 1a). Three groups of subjects were tested. The training sequence (A) was the same for all groups, while the transfer sequence (B) was different between groups (Figure 1 b). The difference in block performance was interpreted as sequence-specific learning (A3 - R3 and B3 - R4) and sequence-specific transfer (B1 - R3 and A4 - R4). **Results:** There was complete transfer of learning when the left and right sequences were shifted to create a new bilateral sequence (Group 1). There also complete transfer of learning when the left and right sequences were switched between legs (Group 2). There was incomplete transfer when the left and right sequences were changed (Group 3). In conclusion, the results observed in the present study suggested that unilaterally sequence components were learned and stored, and that inter-limb transfer learning is mediated at a higher level allowing the task to be executed with either leg.

a



b

Blocks	Groups	Step Length Sequence		
		Left	Right	Bilateral
Training (A1-4)	All	L L M	S M S	S L M L S M
Testing (B1-3)	1	L L M	S M S	S M M L S L
	2	S M S	L L M	L S L M M S
	3	S S L	M L M	M S L S M L

Figure 1 (a) Sequence learning paradigm **(b)** Summary of training and testing sequence - Step length: short (S), medium (M) and long (L).

Disclosures: G. Borin: None. **J.T. Choi:** None.

Poster

722. Posture and Gait: Higher Order Control

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Program#/Poster#: 722.15/VV2

Topic: E.06. Posture and Gait

Support: US Department of Veterans Affairs Rehabilitation Research and Development Service (B1149-R, B9252-C, 0115BRRC-02)

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North Florida/South Georgia Veterans Health System, Gainesville, FL

Florence P. Kendall Post-Professional Doctoral Scholarship, Foundation for Physical Therapy

Title: Executive control of walking in adults with mobility deficits quantified by fNIRS neuroimaging

Authors: *K. A. HAWKINS[1], E. J. FOX[1,5], J. J. DALY[6,2], D. K. ROSE[1,6], E. A. CHRISTOU[3], D. M. OTZEL[7], K. A. BUTERA[1,6], S. A. CHATTERJEE[1], D. J. CLARK[6,4];
[1]Physical Therapy, [2]Neurol., [3]Applied Physiol. and Kinesiology, [4]Aging and Geriatric Res., Univ. of Florida, Gainesville, FL; [5]Clin. Res. Ctr., Brooks Rehabil., Jacksonville, FL; [6]Brain Rehabil. Res. Ctr. of Excellence, [7]Geriatric Res. Educ. and Clin. Ctr., North Florida/South Georgia Veterans Hlth. Syst., Gainesville, FL

Abstract: Background: Control of walking involves a balance between “automatic” (primarily sub-cerebral) and “executive” (cerebral) control strategies. Executive control has a major role in real-time gait adaptations and may also serve an additional compensatory role for control of basic walking if automaticity is impaired. This compensation could compromise the safety of walking because executive control is attention demanding, slow, and susceptible to interference. New insights into the executive control of walking are possible with the use of functional near infrared spectroscopy (fNIRS), which allows for cortical neuroimaging during natural movements. We hypothesize that individuals with mobility deficits will exhibit greater reliance on executive control during walking, as measured by prefrontal cortex activation. Methods: Participants included 14 adults post-stroke with moderate/severe mobility deficits, 15 elderly adults with mild mobility deficits, and 9 young healthy adults. fNIRS was used to measure prefrontal cortex activation during four walking tasks: typical walking, walking over obstacles, walking in dim lighting, and a dual-task condition of walking plus a verbal fluency task. Participants with stroke were also assessed with the Activities Specific Balance Confidence

Scale and the Fugl-Meyer Assessment of lower extremity function. Results: Prefrontal activity during walking showed a main effect of group ($p<0.001$) and task ($p<0.001$), with stroke > elderly > young. Furthermore, lower functioning stroke participants exhibited greater prefrontal activity than higher functioning stroke participants when subgroups were defined by either the Fugl-Meyer score ($p=0.011$) or the balance confidence score ($p=0.006$). Prefrontal/executive reserve capacity, as quantified by the difference in prefrontal activity between dual-tasking and typical walking, was lower in the elderly and post-stroke participants ($p=0.003$). Conclusions: Executive control of typical and complex walking tasks is increased in individuals with mobility deficits, as indicated by greater prefrontal cortical activity. Increased use of executive control is likely a compensation for impairment in neural circuits of locomotor automaticity. Assessing locomotor control strategies with fNIRS is a promising direction for objective assessment of impairment and recovery in humans with mobility deficits.

Disclosures: **K.A. Hawkins:** None. **E.J. Fox:** None. **J.J. Daly:** None. **D.K. Rose:** None. **E.A. Christou:** None. **D.M. Otzel:** None. **K.A. Butera:** None. **S.A. Chatterjee:** None. **D.J. Clark:** None.

Poster

722. Posture and Gait: Higher Order Control

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Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 722.16/VV3

Topic: E.06. Posture and Gait

Support: Imperial College London

Title: Motor cortical modulation of trunk muscles during anticipatory postural adjustments.

Authors: J. QUEK, S.-Y. CHIOU, T. A. CONSTANTIN, *P. H. STRUTTON;
Imperial Col. London, London, United Kingdom

Abstract: The increase in trunk muscle activity prior to rapid movement of the upper limbs is termed anticipatory postural adjustments (APAs). Since APAs occur within a time window considered too fast to be a result of afferent feedback, it has been suggested that they are controlled in part by cortical mechanisms. This is substantiated by our previous work showing increases in corticospinal excitability of trunk muscles at time points closer to the initiation of rapid arm movements. However, the neural mechanisms modulating these increases in corticospinal excitability of the trunk muscles remain unclear, and were therefore investigated in the present study. Twenty healthy subjects performed rapid shoulder flexion in response to a visual cue while standing. Electromyographic (EMG) activity was recorded from the anterior

deltoid (AD) and erector spinae (ES) at the T12 vertebral level. Non-invasive cortical and cervicomedullary stimulation were used to examine motor evoked potentials (MEPs) and the activity of intracortical inhibitory circuits (short-interval intracortical inhibition, SICI) measured from the ES. Stimuli were given at rest, and at two time points (75ms and 25ms) prior to the expected increase in EMG activity of the AD; this was calculated using a recognition reaction time task prior to experimentation. Compared to those at rest, MEPs in the ES were similar at 75ms prior to the increase in AD EMG but were larger at 25ms prior to the increase in AD EMG. In addition, at 75ms, SICI was comparable to that at rest, but at 25ms, was significantly reduced. In contrast, cervicomedullary MEPs in the ES remained unchanged across time points. Our results suggest that the increase in corticospinal excitability of the ES during the APAs are modulated at a cortical level. These findings not only provide insight into the neural mechanisms of APAs in the trunk muscles, but also highlight the importance of using functional task training in subjects with deficits of postural control such as lower back pain and stroke. This may be relevant in the development of rehabilitative strategies for such conditions.

Disclosures: J. Quek: None. **S. Chiou:** None. **T.A. Constantin:** None. **P.H. Strutton:** None.

Poster

722. Posture and Gait: Higher Order Control

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Program#/Poster#: 722.17/VV4

Topic: E.06. Posture and Gait

Title: Localization of cortical and subcortical activity related to reactive balance control with or without performing a visual-working-memory task

Authors: M. D. BOGOST[1], P. I. BURGOS[2], C. E. LITTLE[3], M. WOOLLACOTT[1], *B. H. DALTON[1];
[1]Dept. of Human Physiol., Univ. of Oregon, Eugene, OR; [2]Dept. of Kinesiology, Univ. de Chile, Santiago, Chile; [3]Fac. of Kinesiology, Univ. of Calgary, Calgary, AB, Canada

Abstract: Upright balance equilibrium relies on complex processing and integration of information arising from visual, somatosensory and vestibular cues. However, little is known about the subcortical and cortical structures that are active with respect to reactive postural control. The purpose of this study was to elucidate the active brain areas related to whole-body surface translations and to determine whether the activity of these localized cortical and subcortical areas is depressed during a dual task compared to a single task paradigm. This study utilized high-density electroencephalography in conjunction with independent component analysis, dipole location, and measure projection analysis from event-related potentials (ERPs)

time-locked to backwards surface translation onsets to determine which cortical or subcortical structures were active when performing reactive postural control. Participants (n = 15) either reacted to whole-body surface translations while performing a visual working memory task (dual task) or standing quietly (single task). During the single task, activity was localized to the following sources: somatosensory, motor, spatial processing, conflict and error detecting areas. Interestingly, activity related to the perturbation onset shifted to more somatosensory association, spatial, navigation and memory processing and visual and auditory processing areas and less error detecting areas during the dual task. Further, mean absolute N1 ERP amplitudes from brain areas active during both tasks were significantly attenuated during the dual-task compared to the single-task paradigm. Overall, our results indicate additional frontoparietal area activity, related to spatial attention and memory, and less activation within areas related to error-detection for the dual compared with single task. These results provide evidence of resource re-allocation within the cortical and subcortical structures in the presence of a dual task compared with a single task. Thus, we emphasize here that reactive postural control activity displays distinct patterns within the cortical or subcortical structures depending on the task (i.e., single versus dual) suggesting an attenuation of ERP activity and a re-allocation of neural resources during a dual task when compared to a single task.

Disclosures: **M.D. Bogost:** None. **P.I. Burgos:** None. **C.E. Little:** None. **M. Woollacott:** None. **B.H. Dalton:** None.

Poster

722. Posture and Gait: Higher Order Control

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Program#/Poster#: 722.18/VV5

Topic: E.06. Posture and Gait

Support: NIH 1T32 EY021462

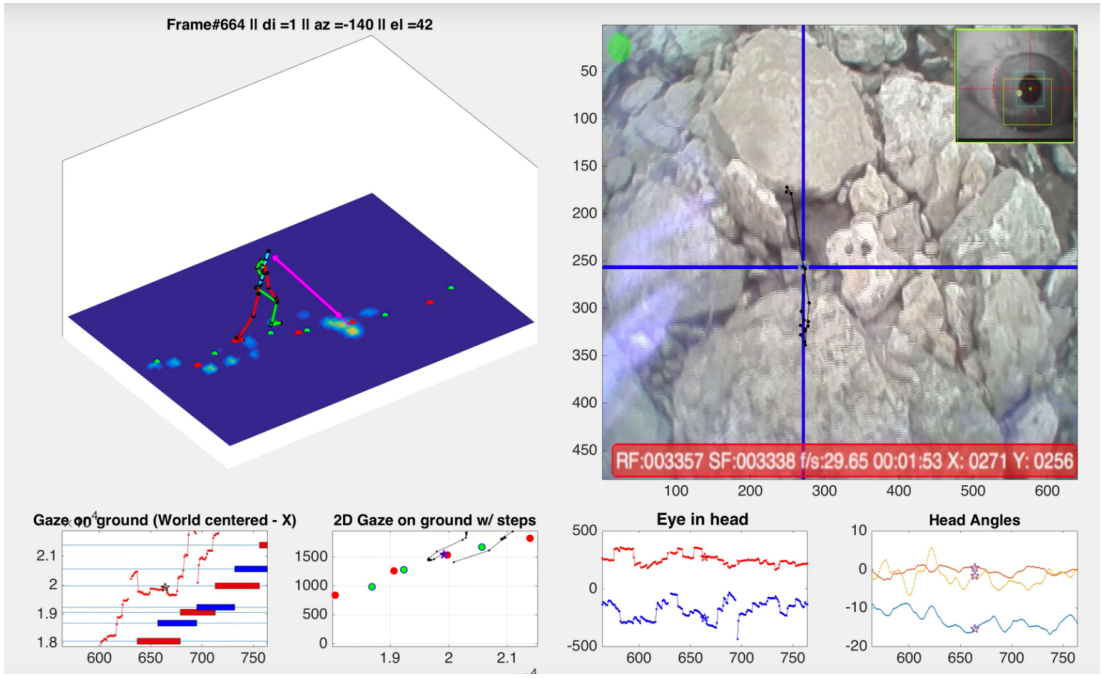
NIH R01 EY05729

Title: The coupling of gaze and gait when walking over real-world rough terrain

Authors: *J. S. MATTHIS;
Ctr. for Perceptual Systems, Res. Univ., Austin, TX

Abstract: When walking over rough terrain, walkers gather information about the upcoming path to support stable and efficient locomotion. In this context, the biomechanics of human gait define the task constraints that organize eye movements when traversing difficult terrain.

Specifically, humans must perform a rapid visual search to identify stable footholds in upcoming terrain in a manner that is temporal coupled to the ongoing gait cycle. In this way, walkers adapt their energetically optimized, biomechanically preferred gait cycle to account for the complexity of the terrain being traversed. We developed a novel experimental apparatus to record eye movements and full-body kinematics of subjects walking over real-world terrain. For the first time, we can precisely record gaze and body movement data during natural behavior in unconstrained outdoor environments. Subjects walked over terrain of three increasing difficulties: flat packed-earth paths, moderately rocky trails, and extremely rough dry creekbeds. In flat terrain, the absence of environmental impediments allows subjects to adopt their preferred gait cycle that exhibits highly regular stride length and timing. In this condition, ground fixations were infrequent and not directed towards footholds, consistent with the minimal visual information needed to guide stepping in flat terrain. When walking over the moderate and extremely rough terrain displayed strong spatiotemporal coupling between gaze patterns on upcoming terrain and their ongoing gait cycle. In difficult terrain, subjects performed rapid visual search on regions around 2-4 steps ahead, often fixating precisely on locations of upcoming footholds. In all types of terrain, both visual and locomotor aspects of subject behavior shows a highly regular phase timing, while the spatial components were more variable. These results highlight the essential inseparability of gaze and gait during locomotion over rough terrain, and indicate that the visual control of locomotion over rough terrain is primarily organized around the phasic timing of the bipedal gait cycle.



Disclosures: **J.S. Matthis:** None.

Poster

722. Posture and Gait: Higher Order Control

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Program#/Poster#: 722.19/VV6

Topic: E.06. Posture and Gait

Support: Grant-in-Aid for Scientific Research (C) (Number 26440265) from the Japan Society for the Promotion of Science

Title: Effects of initial standing position on postural control associated with arm movement and contingent negative variation

Authors: *K. MAEDA[1], K. FUJIWARA[2];
[1]Morinomiya Univ. of Med. Sci., Osaka, Japan; [2]Dept. of Sports and Hlth., Kanazawa Gakuin Univ., Kanazawa, Japan

Abstract: [Purpose] We investigated the effects of difference of initial standing position on postural control associated with bilateral arm movement and contingent negative variation (CNV). [Methods] Thirteen healthy adults (age: 20-42) standing on a force platform rapidly flexed their shoulder bilaterally from the sides of the body to the horizontal level in response to a response signal (S2) in warning signal (S1) - S2 paradigm (inter-stimulus interval (ISI): 2 sec). The standing positions the subjects maintained until S2 presentation (initial positions (IPs)), calculated as the percentage distance of COP from the heel in relation to foot length (%FL), were as follows: quiet standing position, 20%FL, 30%FL, 40%FL, 50%FL, 60%FL, 70%FL and 80%FL. After ten trials of arm movement were performed at any position next ten trials were performed at another position. The order of the position was randomized for each subject. After 10 trials were performed at each position another 10-15 trials were performed for each position. Electromyography (EMG) were recorded from anterior deltoid (AD), erector spinae (ES), biceps femoris (BF), the medial head of the gastrocnemius (GcM), soleus (Sol) and abductor hallucis (AH). Electroencephalography (EEG) recorded from Cz during ISI was averaged for each IP with mean amplitude in the 500 ms period before S1 was used as the baseline. The mean waveforms shifted negatively during ISI were defined as CNV. [Results] Preceding activation with respect to activation of AD was found at all IP in ES, 40%FL and more anterior in BF, 50%FL and more anterior in GcM and 70%FL and 80%FL in AH. Continuous significant increase in CNV from baseline ($p < 0.05$) was found considerably earlier at 70%FL and 80%FL than another position. IP at which significantly larger CNV amplitude with respect to the mean amplitude in the period of 1000-1100 ms before S2 ($p < 0.05$) were found at 50%FL, 60%FL and 80%FL. [Discussion] It was suggested that the onset timing of brain activity related to attention and motor preparation become relatively earlier as the initial standing position shifts near an

extreme forward position and that the preceding activation of the triceps surae was effective in postural control since the activation was found at more anterior position than quiet standing.

Disclosures: **K. Maeda:** None. **K. Fujiwara:** None.

Poster

722. Posture and Gait: Higher Order Control

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Program#/Poster#: 722.20/VV7

Topic: E.06. Posture and Gait

Support: Imperial College London

Title: Effects of hand posture on corticospinal excitability of the trunk muscles in dynamic tasks.

Authors: ***S. CHIDAMBARAM**, J. K. L. WONG, S.-Y. CHIOU, P. H. STRUTTON; Imperial Col. London, London, United Kingdom

Abstract: Trunk muscles play an important role in the maintenance of posture and execution of voluntary movements. It is well established that movements of the upper limbs induce activity in muscles that are related to, but not directly involved in the task. These postural adjustments serve to stabilise the body against the impending perturbation, but the neural mechanisms underlying them are unclear. We have shown that contractions of the upper limb muscles, including those of the hand, increase the corticospinal excitability of trunk muscles. This cross-facilitation effect has been characterised in hand muscles. However, the link between such fine control of the hand muscles and trunk muscles has not been studied. Thus, the present study investigates the effects of hand posture on the corticospinal excitability to trunk muscles using tasks involving power and precision grip.

Twenty healthy subjects [mean (SD) age 22.2 (0.89) years] participated in the study. Subjects were seated and instructed to reach, with the dominant hand, for a wooden ball and a plastic peg, then lift up the object using a power grip or a precision grip, respectively. Electromyographic activity was recorded from the contralateral erector spinae (ES) at vertebral level T12 and rectus abdominis (RA). Transcranial magnetic stimulation (TMS) was delivered over the motor cortex, to the hotspot for ES, at 120% active motor threshold. During the tasks, TMS was delivered while reaching, just prior to the object being picked up, or after it had been picked up. The sizes of the motor evoked potentials (MEPs) were normalised to a control condition (subjects seated with arms extended in front). The amplitudes were calculated to assess the corticospinal excitability of the trunk muscles. Background activity in ES was matched across tasks to minimise the effects of ongoing EMG on the MEP size.

The results showed normalised MEPs were greater in ES when executing the power grip than the precision grip while reaching for the object (141.52±12.2% power vs 106.7±8.87% precision) and upon lifting the object up (156.4±16.7% power vs 122.0±10.3% precision). There was no difference between the precision grip tasks and control tasks or between the "pre" and "post" pickup tasks. The results suggest that there is a task specificity in the corticospinal control of trunk muscles, even during hand movements, which likely induces far less of a postural disturbance than rapid movements of the entire upper limb. Further, the clinical implications of these results include rehabilitation, where improvements in trunk control in subjects with incomplete spinal cord injury, using repetition of simple hand movements, might be possible.

Disclosures: **S. Chidambaram:** None. **J.K.L. Wong:** None. **S. Chiou:** None. **P.H. Strutton:** None.

Poster

722. Posture and Gait: Higher Order Control

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Program#/Poster#: 722.21/VV8

Topic: E.06. Posture and Gait

Support: FAPESP Grant #2012/19943-0

Title: Effects of light touch and visual search on the rambling and trembling components of postural sway

Authors: *D. G. SANTOS[1,2], R. GARBUS[3], S. FREITAS[3];
[1]Lab. do Movimento (LAM), Univ. Cidade De Sao Paulo (UNICID), Sao Paulo, Brazil; [2]Physiotherapy, Hosp. Israelita Albert Einstein, São Paulo, Brazil; [3]Laboratório do Movimento (LAM), Univ. Cidade de São Paulo (UNICID), São Paulo, Brazil

Abstract: The light touch of the index fingertip on a fixed surface provides additional somatosensory information that is used to reduce postural sway during quiet standing. Individuals also reduce their postural sway when performing a cognitive, visual search task while standing. However, it is still unknown which components of the postural sway (i.e., rambling and trembling) are affected by the light touch and visual search task. Therefore, the aim of the present study was to examine the effects of the light touch and visual search task on the components of the postural sway. Thirteen right-handed adults stood, as quiet as possible, on an AMTI force plate during 70 seconds. Participants performed three trials for each touch (with and without the right index fingertip touching a fixed bar composed by a force sensor ATI) and visual (with or without visual search task) conditions. For the visual search condition,

participants were instructed to search and count the letters in a text displayed one meter ahead and to say the number of letters counted to the evaluator after each trial. Different letters were used for each trial. For touch condition, participants maintained the elbow in extension while lightly touched (applied force less than 1 N) a rigid surface using their right index fingertip. The center of pressure (COP) at anterior-posterior direction was computed using the forces and moments of the force plate and decomposed in two components: rambling, representing the migration of the reference point on the supporting surface, and trembling, representing the deviation of the COP from its equilibrium position. The amplitude of COP and its components, the mean force applied on the bar during the trials with touch and the error in the cognitive task were assessed. The COP and rambling amplitude reduced when participants performed the visual search task or touched the bar. However, the trembling reduced only in the touch condition with or without the visual search task being performed simultaneously. For all conditions, the reduction of the rambling was greater compared to the trembling. The force applied on the bar increased when the search task was performed with the touch but the performance on the cognitive task was not affected by the touch. The visual search task affects only the rambling trajectory that reflects the supraspinal process. Conversely, the light touch reduces, in addition to the rambling, the trembling trajectories that reflect the action of spinal reflexes and the mechanical properties of the muscles and joints. Overall, the findings suggest that the reduction on postural sway is mainly due to the need of postural stabilization to perform the visual search task or the touch.

Disclosures: **D.G. Santos:** None. **R. Garbus:** None. **S. Freitas:** None.

Poster

722. Posture and Gait: Higher Order Control

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Eric P. and Evelyn E. Newman Fund

Title: Dynamic entrainment of human walking to ankle dorsi-flexion perturbations

Authors: *J. OCHOA[1], D. STERNAD[2], N. HOGAN[3];
[1]Mechanical Engin., Massachusetts Inst. of Technol. Dept. of Mechanical Engin., Cambridge, MA; [2]Biology, Electrical and Computer Engineering, and Physics, Northeastern Univ., Boston, MA; [3]Mechanical Engineering, and Brain and Cognitive Sci., MIT, Cambridge, MA

Abstract: The concept of a central pattern generator as a rhythmic movement primitive has been well established in animal locomotion. Our recent research on human locomotion demonstrated dynamic entrainment to periodic perturbations in ankle plantar-flexion, evidence of an underlying limit-cycle oscillator. *Entrainment* of subjects' gaits with *phase-locking* at ankle 'push-off' suggested that the torque pulses provided mechanical assistance with forward propulsion. To further explore the mechanical and neural origin of that oscillator, this study tested the effect of torque pulses in dorsi-flexion given that 'toe-up' torque does not assist propulsion. As before, perturbations were delivered periodically to both treadmill and overground locomotion.

Fourteen healthy subjects walked overground (OG) and on a treadmill (TM) while wearing an exoskeletal robot that was programmed to exert short square torque pulses in dorsi-flexion at a period 50 ms different from subjects' preferred stride period. Entrainment was observed during both OG and TM walking, evidenced by a near-constant phase of maximum knee flexion with respect to the torque pulse. Entrainment during TM trials took longer in comparison to OG trials, occurring after an average of 15 vs. 26 perturbation cycles, respectively. Entrainment was always accompanied by phase-locking: the pulses converged to 'initial swing' (71.92% ± 4.28% of the gait cycle), suggesting that subjects assumed a phase relation such that the perturbation assisted ankle dorsi-flexion to facilitate foot-ground clearance and limb advancement.

These observations support the presence of a limit-cycle attractor underlying human locomotion. However, these results cannot be attributed to mechanics alone since phase-locking occurred while the foot was in the air, when the imposed torque pulses did not influence mechanical work. Overall, the observed behavior seems to require a neural adaptation that cannot easily be ascribed to biomechanics, suggesting a hierarchical organization between the supra-spinal nervous system and the spinal neuro-mechanical periphery: *episodic supervisory control*. In this scenario, the supra-spinal nervous system may behave as a *tele-operator* of the *semi-autonomous* spinal neuro-mechanical periphery to reduce the burden on higher centers of the brain, especially given the delays in neural conduction and limited speed of muscle response. Taken together, these results indicate that a limit-cycle oscillator exists in the semi-autonomous lower-levels, which is capable of stable rhythmic walking with little high-level intervention.

Disclosures: J. Ochoa: None. **D. Sternad:** None. **N. Hogan:** None.

Poster

722. Posture and Gait: Higher Order Control

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Topic: E.06. Posture and Gait

Support: NASA Grant NCC 9–58

NIH Grant RO1-DC009031

Title: Estimation of optimal stimulus amplitude for balance training using electrical stimulation of the vestibular system

Authors: *R. GOEL[1], M. J. ROSENBERG[2], Y. E. DEDIOS[3], H. S. COHEN[2], J. J. BLOOMBERG[4], A. P. MULAVARA[5];
[1]Univ. of Houston, Houston, TX; [2]Baylor Col. of Med., Houston, TX; [3]Wyle Science, Technol. and Engin. Group, Houston, TX; [4]NASA Johnson Space Ctr., Houston, TX; [5]Universities Space Res. Assn., Houston, TX

Abstract: Sensorimotor changes such as postural and gait instabilities can affect the functional performance of astronauts following gravitational transitions. By training astronauts preflight with supra-threshold noisy stochastic vestibular stimulation (SVS), the central nervous system can be trained to reweight sensory information by utilizing veridical information from other sensory inputs, such as vision and proprioception, for postural and gait control. This, in turn, can enhance functional performance in novel gravitational environments. The optimal maximum amplitude of stimulation to simulate the effect of deterioration in vestibular inputs for preflight training or for evaluating vestibular contribution in functional tests in general, however, has not yet been identified. Most studies have used arbitrary but fixed maximum current amplitudes from 3 to 5 mA in the medio-lateral (ML) direction to disrupt balance function in both ML and anterior-posterior (AP) directions in healthy adults. The goal of this study was to determine the minimum SVS level that yields an equivalently degraded balance performance. Fourteen subjects stood on a compliant surface with their eyes closed and were instructed to maintain a stable upright stance. Measures of stability of the head, trunk, and whole body were quantified in the ML direction. Objective perceptual motion thresholds, were estimated ahead of time by having subjects sit on a chair with their eyes closed and giving 1 Hz bipolar binaural sinusoidal electrical stimulation at various current amplitudes. Results from the balance task suggest that using stimulation amplitudes of 280% of motion-perceptual threshold (~2.2 mA on average) significantly degraded balance performance and increasing the stimulation amplitude did not lead to further degradation. We anticipate that preflight training using supra-threshold SVS stimulation will be a component of preflight sensorimotor adaptability (SA) training designed to improve adaptability to novel gravitational environments. This combination may help to

significantly reduce the time to recover functional performance after long-duration spaceflight or after landing in a novel gravitational environment (e.g. Moon or Mars). Another application of using electrical stimulation of the vestibular system is in the evaluation of tests for vestibular function by simulating acute deterioration of vestibular sensory inputs.

Disclosures: R. Goel: None. **M.J. Rosenberg:** None. **Y.E. DeDios:** A. Employment/Salary (full or part-time): Wyle Science, Technology and Engineering Group provided support in the form of salary for author YD but did not have any additional role in the study design, data collection and analysis.. **H.S. Cohen:** None. **J.J. Bloomberg:** None. **A.P. Mulavara:** None.

Poster

723. Spinal Reflexes

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 723.01/VV11

Topic: E.06. Posture and Gait

Title: A comparison of spinal and motor cortical neurophysiology in highly trained martial artists versus untrained controls

Authors: *Y. LIU[1,2], W. OGLE[1], A. M. PHIPPS[1,2], M. R. ENYART[1,2], A. RECTOR[1], D. M. KOCEJA[1,2], H. J. BLOCK[1,2];
[1]Dept. of Kinesiolgoy, [2]Program in Neurosci., Indiana Univ. Bloomington, Bloomington, IN

Abstract: The practice of martial arts requires an exceptional level of control in order to execute motor skills such as overhead kicks with both speed and accuracy while maintaining balance and postural control. Motor skill training has been shown to induce changes in both spinal and cortical neurophysiology. For example, athletes in sports with high postural demands show reduction in spinal Hoffmann reflex (H-Reflex) excitability (Nielsen et al. 1993). In addition, professional musicians show evidence of greater recruitment of neurons in the hand area of motor cortex for a given stimulus intensity (Rosenkranz et al. 2007). These and other studies suggest that expert motor skill can be associated with changes in both spinal and motor cortical circuitry. However, because these sites have not been compared directly in a single highly skilled population, the degree to which each contributes is unknown. Here we asked whether highly trained martial artists would show differences from untrained controls in spinal reflexes, motor cortical responses, or both. To quantify spinal neurophysiology, the H-reflex for the right soleus muscle was measured at varying stimulus intensities to construct a recruitment curve. To quantify motor cortical neurophysiology, motor evoked potentials (MEPs) were elicited with transcranial magnetic stimulation (TMS) applied over the soleus area of the motor cortex; stimulation intensity was varied to construct a recruitment curve for motor cortex. In each case,

the subject was seated and muscle twitch amplitude was measured with electromyography using surface electrodes. Linear regression was used to determine the slope of each recruitment curve. Five right-footed subjects have participated to date: 2 untrained controls and 3 martial artists with 7-15 years of intensive martial art training experience. In these subjects, recruitment curve slope is lower in martial artists than untrained controls for both H-reflex and motor cortex. This could indicate that martial arts training is associated with recruitment of fewer neurons for a given stimulus intensity, at both the cortical and spinal level. Data collection is ongoing, but differences in both cortical and spinal neurophysiology would be consistent with the literature that has found, separately, changes at each level related to highly trained motor skill.

Disclosures: Y. Liu: None. **W. Ogle:** None. **A.M. Phipps:** None. **M.R. Enyart:** None. **A. Rector:** None. **D.M. Koceja:** None. **H.J. Block:** None.

Poster

723. Spinal Reflexes

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 723.02/VV12

Topic: E.06. Posture and Gait

Title: Effects of contralateral common peroneal nerve conditioning on ipsilateral soleus H-reflex during walking

Authors: *M. R. ENYART[1,2], K. KITANO[3], A. M. PHIPPS[2,3], D. M. KOCEJA[3,2];
[2]Program in Neurosci., [3]Kinesiology, [1]Indiana Univ., Bloomington, IN

Abstract: The soleus spinal stretch reflex is implicated in maintaining posture and balance during bipedal human locomotion. Furthermore, previous studies have demonstrated that the neural circuitry from the contralateral antagonist has crossed-spinal modulatory influences on this reflex while standing and lying prone. The influence of the contralateral antagonist has not, however, been investigated during walking. The purpose of this study was to assess the modulatory effect of the contralateral tibialis anterior on the ipsilateral soleus H-reflex. The four subjects who participated were asked to walk at a speed of 4km/hr while receiving tibial nerve stimulations at 7 different interstimulus intervals that represent different times during single leg stance (0, 2.5, 5, 7.5, 10, 12.5, and 15%). These stimulations were delivered at specific intervals following heel strike that corresponded to the desired timing of the stance phase. The conditioning stimuli consisted of trains of 4 stimulations to the common peroneal nerve of the contralateral tibialis anterior spaced 5ms apart, for a total of 20ms. The timing of the conditioning stimuli were fixed to 30% of the gait cycle following ipsilateral heel strike, and the intensity was set to 1.5x motor threshold. Intensity for the control soleus H-reflex was set to 15%

of M-max for each subject, which was obtained during walking. The conditioning protocol resulted in facilitation of the ipsilateral soleus H-reflex at the 7.5% interstimulus interval, which was significantly different from the control value ($t(3) = 3.780$, $p = .032$). The results demonstrate that at an interstimulus interval of 7.5% of single leg stance (~80ms following conditioning stimulus), the modulatory effect of the contralateral tibialis anterior on the ipsilateral soleus is facilitation. This is consistent with the literature that exists on standing subjects, which shows a facilitatory effect of the contralateral tibialis anterior at an interstimulus interval of 75ms. Further research is needed to elucidate the effects of the contralateral agonist muscle on the ipsilateral agonist (i.e. soleus-soleus interaction).

Disclosures: **M.R. Enyart:** None. **K. Kitano:** None. **A.M. Phipps:** None. **D.M. Koceja:** None.

Poster

723. Spinal Reflexes

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 723.03/VV13

Topic: E.06. Posture and Gait

Support: Wellcome Trust

Title: Response of spinal cord interneurones to finger perturbations.

Authors: *D. S. SOTEROPOULOS, S. BAKER;
Newcastle Univ., Newcastle Upon Tyne, United Kingdom

Abstract: For hand and wrist muscles there is strong evidence demonstrating that the primary motor cortex is a major contributor to the long latency response seen in muscles following a mechanical perturbation. There are also other areas that are likely to contribute, but the contribution (if any) from spinal cord (SC) circuits has so far been rarely considered. Two adult awake behaving monkeys were trained to perform a precision flexion finger movement with the index finger. At the end of each trial, the motor controlled lever returned the finger to starting position rapidly, with peak velocities in excess of 200^{o} per second. This produced a response in muscles controlling the finger, recorded through intramuscular wire electrodes. During performance of this task we recorded the extracellular activity of neurones firstly from the contralateral M1 (n=211) and subsequently from the ipsilateral intermediate zone SC (n=119). The perturbation produced a response in over 60% of neurones in both M1 (149/211) and SC (76/119). Comparable fractions of identified neurones responded in each area - 73% of corticospinal cells (n=73) in M1, and 62 of premotor spinal interneurons (n=21) responded to the perturbation. In M1, of the cells responding to the perturbation, 67% did so with an increase in

firing rate, while in the spinal cord this fraction was significantly ($p<0.01$, X^2 test) much higher at 82%. The onset latency of the neural responses however were comparable between the two areas (M1 mean: 20.6ms, SC mean: 20.2ms, $p>0.2$, unpaired t-test). The amplitude of the response to the perturbation relative to the baseline was also comparable between the two areas (M1 mean: 1.6, SC mean: 1.6, $p>0.4$, unpaired t-test). Our results support the well-established involvement of M1 in long latency reflexes, but also support a contribution from spinal cord interneurons to both short and long latency components of the stretch response.

Disclosures: D.S. Soteropoulos: None. **S. Baker:** None.

Poster

723. Spinal Reflexes

Location: Halls B-H

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Program#/Poster#: 723.04/VV14

Topic: E.06. Posture and Gait

Support: NIH R01 AR-050520

NIH R01 AR-052345

FRQNT 190307

USC Provost Fellowship

Title: Changes in fusimotor parameters suffice to explain position thresholds, velocity thresholds, and gains of the stretch reflex; but produce strong interactions among them

Authors: *K. JALALEDDINI[1], A. MARJANINEJAD[2], S. CHAKRAVARTHI RAJA[3], F. J. VALERO-CUEVAS[1,2];
[1]Div. of Biokinesiology and Physical Therapy, [2]Dept. of Biomed. Engin., [3]Ming Hsieh Dept. of Electrical Engin., USC, Los Angeles, CA

Abstract: Position threshold, velocity threshold, and gain are the preferred kinematic and EMG metrics to assess the strength of the stretch reflex. The thresholds describe how soon the reflex is evoked and the gain describes the sensitivity of the response. But how do these metrics arise from changes in physiological parameters of the monosynaptic stretch reflex such as of dynamic and static fusimotor drives? We used a neuromorphic system to answer this question. The system was a faithful implementation of the spinal stretch reflex circuitry with a population of spiking neurons, including 128 muscle spindles with tunable dynamic and static fusimotor drives and 768 motor neurons with rate coding and size principle. They controlled the input of a Hill-type

model of skeletal muscle to emulate the muscle force. An electric motor converted the emulated muscle force to tension in a tendon. Finally, two independent systems emulated the forces of a pair of antagonistic tendons, acting to the flexion-extension of the joint. We performed ramp-and-hold perturbations to the joint (replicating those performed in the assessment of the stretch reflex) using a stiff servo motor with a wide range of velocities while systematically and differentially changing the static and dynamic fusimotor drives. For each set of fusimotor parameters, we quantified the minimum amplitude and velocity of perturbation required to evoke a reflex as well as the gain of the response.
We find that 1) changes in fusimotor drive resulted in a wide range of thresholds and gain within the reported range for normal and pathological populations, and 2) these metrics were correlated to each other and depended on the values of the static and dynamic fusimotor drives. This demonstrates that our neuromorphic system sufficed to emulate healthy and pathologic behaviors. We conclude that measurement of the thresholds together with gain is necessary to fully explain the strength of the stretch reflex response. Moreover, our results inform new approaches to use simple reflex tests to interrogate the underlying mechanisms of fusimotor control.
Research reported in this publication was supported by the National Institute of Arthritis and Musculoskeletal and Skin Diseases of the National Institutes of Health under Awards Number *R01 AR-050520* and *R01 AR-052345,* by the Fonds de recherche du Québec - Nature et technologies, and by USC provost fellowship. The contents of this endeavor is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Disclosures: K. Jalaleddini: None. **A. Marjaninejad:** None. **S. Chakravarthi Raja:** None. **F.J. Valero-Cuevas:** None.

Poster

723. Spinal Reflexes

Location: Halls B-H

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Topic: E.06. Posture and Gait

Support: NIH Grant R01 NS053813

NIH Grant T32 HD07418

Title: Stretch reflexes in shoulder muscles are described best by heteronymous pathways

Authors: *M. H. SOHN[1,2], E. M. BAILLARGEON[1,2], D. B. LIPPS[3], E. J. PERREAULT[1,2]; [1]Northwestern Univ., Chicago, IL; [2]Sensory Motor Performance Program, Rehabil. Inst. of Chicago, Chicago, IL; [3]Univ. of Michigan, Ann Arbor, MI

Abstract: Healthy shoulder function requires the humeral head to remain secure in the glenoid cavity. Stretch-sensitive feedback may be critical for glenohumeral stability, since it is known that pathologies impairing feedback (e.g. stroke or spinal cord injury) often lead to shoulder instability. However, little is known about stretch reflexes at the shoulder due to experimental limitations. This study investigated the coordination of stretch reflexes elicited by rotations of the glenohumeral joint using a manuipulandum developed by our lab to study 3D shoulder mechanics and neural control. We hypothesized that stretch reflexes would be described more accurately by a heteronymous model linking muscles of the shoulder than by a homonymous model considering only the state of an individual muscle. Subjects (n=10) were seated with the right arm fixed to a rotary motor. Electromyograms (EMGs) were recorded from 8 shoulder muscles in response to pseudo-random binary perturbations as subjects exerted voluntary isometric torques (±10 and ±20% max contraction) about 3 measurement axes. Reflexes were quantified by average rectified EMG in short- (20~50 ms), medium- (50~75 ms), and long-latency windows (75~100 ms) after perturbation onset. Background activity was computed 100 ms before each perturbation. The volitional torques created rich patterns of background activity, providing a means to test our hypothesis using a linear mixed-effects model between background activity and stretch reflexes. Separate models (n=144) were constructed for reflexes in each muscle, perturbation direction, and response window. A simulated log-likelihood ratio was used to determine if models considering background activity in all muscles (heteronymous) were significantly better than those considering only background activity in the muscle in which the reflex was measured (homonymous). The heteronymous model was significantly better than the homonymous model in all tested conditions. The heteronymous models had an average $R^2 = 0.68 \pm 0.19$, and explained 28.6 ± 22.5% (mean ± std) more of the total measured variance than the homonymous models, demonstrating a wide range of improvements. The improvement differed across response windows ($p = 0.025$; 1-way ANOVA): greater ($p = 0.013$; Tukey's correction) in the long-latency (34.5 ± 3.45%, mean ± se) than the short-latency (22.1 ± 3.10%) window. Our results demonstrate that stretch reflexes in the shoulder are sensitive to changes in the background activity of multiple muscles, not simply that from which the reflex is measured. This suggests that shoulder reflexes consider the state of the entire joint to coordinate a response to perturbations.

Disclosures: M.H. Sohn: None. **E.M. Baillargeon:** None. **D.B. Lipps:** None. **E.J. Perreault:** None.

Poster

723. Spinal Reflexes

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Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 723.06/VV16

Topic: E.06. Posture and Gait

Title: Implementation of a multilayer perceptron neural network for classifying a hemiplegic and healthy reflex pair using an iPod wireless gyroscope platform

Authors: *R. C. LEMOYNE[1], T. MASTROIANNI[2];
[1]Independent, Running Springs, CA; [2]Independent, Pittsburgh, PA

Abstract: The features of the patellar tendon reflex response provide significant perspective for the standard neurological examination. The iPod is a ubiquitous portable media device that has successfully quantified the patellar tendon reflex response. The iPod is equipped with inertial sensors, such as a gyroscope, which enable a convenient representation of the patellar tendon reflex response. The iPod can function as a wireless gyroscope platform by the incorporation of a software application. The integration of a multilayer perceptron neural network to distinguish a feature set of the patellar tendon reflex response provides expanded capability of the system incorporating the iPod as a wireless gyroscope platform for reflex quantification. Machine learning platforms, such as the multilayer perceptron neural network, offer considerable utility relative to the standard neurological examination. A hemiplegic and healthy patellar tendon reflex pair demonstrates visibly distinguishable characteristics, which can be consolidated into a quantified feature set from a gyroscope signal recording. The iPod utilizes a software application to record the gyroscope signal and wirelessly transmit the trial as an email attachment for post-processing amenable to machine learning. The iPod wireless gyroscope platform implementing a multilayer perceptron neural network successfully classifies a hemiplegic and healthy reflex pair.

Disclosures: R.C. LeMoyne: None. **T. Mastroianni:** None.

Poster

723. Spinal Reflexes

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 723.07/VV17

Topic: E.06. Posture and Gait

Support: NSERC Discovery Grant

Title: Static and dynamic soleus stretch reflex responses in standing humans are independently modulated by postural threat.

Authors: *B. C. HORSLEN[1], M. ZABACK[1], T. INGLIS[1,3,4], J.-S. BLOUIN[1,4,2], M. G. CARPENTER[1,3,4];
[1]Sch. of Kinesiology, [2]The Inst. for Computing, Information and Cognitive Systems, Univ. of British Columbia, Vancouver, BC, Canada; [3]Intl. Collaboration On Repair Discoveries, Vancouver, BC, Canada; [4]Djavad Mawafaghian Ctr. for Brain Hlth., Vancouver, BC, Canada

Abstract: Threats to standing balance have been shown to increase tendon-tap stretch (T-) reflexes, likely due to increased muscle spindle sensitivity [1]. Spindle static and dynamic sensitivity are thought to change differently with task novelty or threat in cats [2], yet T-reflexes are primarily subject to dynamic sensitivity, and involve only a select number of receptors. As such, it is unknown if threat independently modulates static and dynamic aspects of spindle-based reflex responses to large muscle stretches in humans. This study examined velocity- and amplitude-dependent changes to spindle-based ramp-and-hold short- (SLR) and medium-latency stretch reflexes (MLR) with a height-induced postural threat.
Sixteen young healthy adults stood with their right foot on a servo-controlled tilting platform and left foot on a stable surface level with the tilting platform; the platform was positioned at the edge of a hydraulic lift. Participants stood at a low threat condition (1.1m high; away from edge) followed by a high threat condition (3.5m high; at edge). Toe-up ramp-and-hold stretches were delivered at 3 angular velocities (Vel) of 60, 100 and 170°/s (all to 5°), and 3 amplitudes (Amp) of 2, 5 and 7° (all at 170°/s); Soleus T-reflexes were evoked by taps to the Achilles tendon with a linear motor. Surface EMG was recorded from Soleus and Tibialis Anterior, and platform Amp and Vel were calculated from a potentiometer. Individual slopes were calculated between SLR and platform Vel (dynamic) and MLR and platform Amp (static) and compared across heights. Main effects of height were also calculated for SLR and MLR amplitudes.
T-reflexes were on average 14% larger at height ($p=0.06$), replicating prior work [1]. SLR and MLR amplitudes were correlated to platform Vel and Amp, respectively (SLR low: $r=0.49$; SLR high: $r=0.57$; MLR LOW: $r=0.80$; MLR HIGH: $r=0.79$). SLR/Vel slopes tended to be steeper at height (44% steeper, $p=0.067$), yet MLR/Amp slopes were not affected by height (7% steeper, $p=0.5$). There were no main effects of height on either SLR (5% larger, $p=0.52$) or MLR amplitudes (3.5% larger, $p=0.34$).
Observations of increased velocity-dependent scaling of human lower-limb SLR slopes and T-reflex amplitudes suggest spindle dynamic sensitivity may be increased with postural threat. The lack of amplitude-dependent MLR slope changes suggests no change in static sensitivity with threat. These results together may be explained by selective fusimotor control, which has been inferred from other human studies using non-balance lower-limb attention tasks [3].
Funded by NSERC.
1-Horslen et al (2013) J Neurophysiol, 2-Prochazka (1989) Prog Neurobiol, 3-Hospod et al (2007) J Neurosci

Disclosures: **B.C. Horslen:** None. **M. Zaback:** None. **T. Inglis:** None. **J. Blouin:** None. **M.G. Carpenter:** None.

Poster

723. Spinal Reflexes

Location: Halls B-H

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Program#/Poster#: 723.08/VV18

Topic: E.06. Posture and Gait

Support: NIH Grant R01 AR-050520

NIH Grant R01 AR-052345

Title: A neuromorphic implementation of spinal alpha-gamma circuity suffices to produce simple voluntary movements in cadaveric hands

Authors: ***S. CHAKRAVARTHI RAJA**[1], K. JALALEDDINI[2], N. LIGHTDALE-MIRIC[4], F. VALERO-CUEVAS[2,3];
[1]Dept. of Electrical Engin., [2]Dept. of Biokinesiology and Physical Therapy, [3]Dept. of Biomed. Engin., USC, Los Angeles, CA; [4]Children's Hosp. Los Angeles, Los Angeles, CA

Abstract: The roles of fusimotor drive and α-γ coactivation in the control of voluntary movement have been debated since the time of Sherrington. We have taken a synthetic analysis approach to build a custom, real-time neuromorphic system which features a physiologically faithful representation of the stretch reflex loop including muscle spindles with tunable fusimotor drives and populations of motor neurons with recruitment and rate-coding [1,2]. This multiscale system allows the recording and adjustment of time-varying physiological variables which are otherwise inaccessible in intact subjects and specimens. The neuromorphic system has recently been used by us to explore the role of fusimotor activity in closed loop monosynaptic stretch reflex responses.
In this work, we demonstrate how the settings of the fusimotor system affect the kinematics that arise from a same sinusoidal alpha command. As a first step, we applied half-wave rectified cortical voluntary drives (i.e., alpha signal) to a pair of antagonist muscles. A 180° phase shift ensured that the drives to the antagonist pair were out of phase and never countered each other, ensuring smooth sinusoidal movement. Upon systematically varying the tonic gains of the fusimotor drives, we found that they affected the amplitude, velocity and mean position of voluntary finger oscillation. To our knowledge, this is the first neuromorphic implementation of voluntary finger movement in cadaver specimens. While these results are not unexpected, they are a critical first step in the simulation and understanding of healthy and pathological

movement, tremor, dystonia, and spasticity. Moreover, this provides a mechanistic platform to explore the role of α-γ coactivation from physiological and anatomical first principles.
Research reported in this publication was supported by the National Institute of Arthritis and Musculoskeletal and Skin Diseases of the National Institutes of Health under Awards Number *R01 AR-050520* and *R01 AR-052345*. The contents of this endeavor is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.
[1] Niu, Chuanxin et al. "Emulated muscle spindle and spiking afferents validates VLSI neuromorphic hardware as a testbed for sensorimotor function and disease." *Frontiers in computational neuroscience*, 2013.
[2] Sohn, Won J. et al. "Increased long-latency reflex activity as a sufficient explanation for childhood hypertonic dystonia: a neuromorphic emulation study." *Journal of neural engineering,* 2015.

Disclosures: S. Chakravarthi Raja: None. **K. Jalaleddini:** None. **N. Lightdale-Miric:** None. **F. Valero-Cuevas:** None.

Poster

723. Spinal Reflexes

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 723.09/VV19

Topic: E.06. Posture and Gait

Title: Soleus H-reflex modulation by the contralateral common peroneal nerve stimulation during the late stance phase of human gait

Authors: *K. KITANO[1], A. PHIPPS[1,2], M. ENYART[1,2], D. KOCEJA[1,2];
[1]Dept. of Kinesiology, [2]Program in Neurosci., Indiana Univ., Bloomington, IN

Abstract: H-reflex methodology and related techniques have been employed in order to study spinal mechanisms. However, neural mechanisms which control bilateral coordination of the extremities are still unclear. Previous studies from our group have demonstrated a possible neural connection between motor pools in the ipsilateral soleus and the contralateral tibialis anterior, which may contribute to the generation of stereotypical bilateral coordination. However, neural mechanisms which embody bilateral coordination is yet to be determined. It is known that human soleus H-reflex is modulated during gait and this modulation is known to be dependent on a phase of the gait cycle. Therefore, the purpose of this study was to explore soleus H-reflex modulation using contralateral tibialis anterior conditioning. Four subjects participated, each with no history of neuromuscular disease and with normal fitness levels. Subjects walked on a

treadmill at 4km/h. An EMG signal was collected from the right soleus and stimulating electrodes were prepared at the right popliteal fossa and the left head of fibula in order to delivery electrical stimulation to the right tibial nerve and the left common peroneal nerve. Control H-reflex amplitudes were set at 15% of M-max and inter-stimulus intervals (ISI) were selected at 0%, 2.5%, 5%, 7.5%, 10%, 12.5%, and 15% of gait cycle. Conditioning stimulation to the left common peroneal nerve was delivered prior to the test stimulation. Conditioning stimuli were a train of 4 pulses during 20 msec and intensity was set at 1.5 motor threshold in the tibialis anterior. Test H-reflex was evoked at two different gait cycle points: single support and double support during the late stance phase. Soleus H-reflex during double support phase was significantly inhibited at 2.5% ISI conditioning (-57%) and significantly facilitated at 10% ISI conditioning (+96%), compared with unconditioned H-reflex. This modulation observed in double support phase was different from that in single support in the late phase. Results suggest that the status at the spinal neural network in the late phase of gait may differ between double support and single support. Sensory inputs attributable to the conditioning stimulation had greater impact on the contralateral soleus H-reflex in double support phase than in single support phase. It is implied that neural inputs from the contralateral side are controlled with other sensory information and/or a phase of gait cycle.

Disclosures: **K. Kitano:** None. **A. Phipps:** None. **M. Enyart:** None. **D. Koceja:** None.

Poster

723. Spinal Reflexes

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Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 723.10/VV20

Topic: E.06. Posture and Gait

Support: NSERC

Title: Interlimb reflexes between the forelimbs and hindlimbs are asymmetrically organized during locomotion in intact adult cats.

Authors: ***M.-F. HURTEAU**, E. DESROCHERS, Y. THIBAUDIER, C. DAMBREVILLE, A. TELONIO, A. FRIGON;
pharmacologie-physiologie, Univ. De Sherbrooke, Sherbrooke, QC, Canada

Abstract: Locomotion requires precise coordination between the four limbs, which is thought to be mediated by pathways coupling the fore- and hindlimbs, such as interlimb reflexes. Miller et al. (1977) showed that homolateral interlimb reflexes in decerebrate cats were modulated with phase and were stronger for descending (forelimb to hindlimb) than ascending (hindlimb to

forelimb) ones. Here, we characterized interlimb reflexes in intact cats during treadmill locomotion and extended the analysis to diagonal limbs. Three cats were implanted with electrodes for electromyography and to stimulate the superficial peroneal (SP) and superficial radial (SR) nerves. Reflexes were evoked during treadmill locomotion at 0.4 m/s with a train of three 0.2 ms pulses at 300 Hz. For each nerve, ~120 stimuli were given at 1.2 times the motor threshold throughout the cycle. Stimulating the SP nerve evoked ascending interlimb reflexes in the homolateral triceps brachii (TriB) in 3/3 cats, with short-latency inhibitory responses (N1) during forelimb stance starting at ~25 ms, followed by small excitatory responses (P2) at ~50 ms. Similar responses were observed in the diagonal TriB of 2/3 cats, with N1 responses during forelimb stance starting at ~20 ms followed by P2 responses at ~45 ms. Ascending interlimb reflexes in the homolateral and diagonal biceps brachii were only observed in 1/3 cats for both limbs, with P1 responses starting at ~20-25 ms during forelimb swing. Therefore, ascending interlimb reflexes appear more frequent in elbow extensors compared to elbow flexors. With SR nerve stimulation, descending interlimb reflexes were evoked in an homolateral hindlimb extensor muscle, vastus lateralis or lateral gastrocnemius, in 2/3 cats, with N1 responses starting at ~30 ms during hindlimb stance. One cat also showed P1 responses starting at ~20 ms during hindlimb swing. Diagonal responses in hindlimb extensors were observed in 3/3 cats, with N1 responses during hindlimb stance starting at ~30 ms. Descending interlimb reflexes were found in the homolateral and diagonal semitendinosus in 3/3 cats, with P1 responses starting at 20-30 ms during hindlimb swing. Therefore, both ascending and descending interlimb reflexes are present during treadmill locomotion in the intact cat, with projections to homolateral and diagonal limbs. Consistent with previous findings, the strength of descending interlimb reflexes appears stronger than ascending ones. Interlimb reflexes could be a mechanism to rapidly reduce the excitability of extensor muscles, thus lowering the center of gravity, and/or to prolong or delay phase transitions to optimize dynamic stability.

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Poster

723. Spinal Reflexes

Location: Halls B-H

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Program#/Poster#: 723.11/VV21

Topic: E.06. Posture and Gait

Title: The roles of contralateral conditioning and ipsilateral control stimulus timing on the soleus H-reflex during human gait

Authors: *A. M. PHIPPS[1,2], K. KATANO[1], M. ENYART[1,2], D. M. KOCEJA[1,2];
[1]Kinesiology, [2]Program in Neurosceince, Indiana Univ. Bloomington, Bloomington, IN

Abstract: Past studies have demonstrated the modulatory effects of crossed-spinal conditioning on the soleus H-reflex. These studies have only explored crossed-spinal conditioning during static positions. However, given the critical role for coordination between the ipsilateral and contralateral muscles of the lower extremities play in movement control, the purpose of this study was to assess crossed-spinal conditioning of the soleus H-reflex during gait. Four young, healthy subjects were tested while walking on a treadmill at 4 km/h. Control H-reflexes, set to 50% Hmax, were elicited from the ipsilateral soleus muscle at two points in the gait cycle: (1) mid stance and (2) late stance. Mid stance was determined to be 30% of the gait cycle, while late stance was determined to be approximately 43% of the gait cycle post-ipsilateral heel strike. Conditioning stimuli, a train of four for 20 ms, was applied to the contralateral common peroneal nerve at the fibular head preceding the ipsilateral test stimulation to the tibial nerve popliteal fossa. Conditioning stimuli were set to 1.5 x motor threshold and delivered at interstimulus intervals (ISIs) set at 0%, 2.5%, 5%, 7.5%, 10%, 12.5%, and 15% of the gait cycle. Contralateral conditioning delivered at 2.5% ISI of the gait cycle resulted in modulation of the soleus H-reflex during late stance phase by -38% (inhibition). A one sample t-test showed this inhibition to be significantly different from the control H-reflex. However, there was no significant inhibition/facilitation of the soleus H-reflex during mid stance phase. These results show contralateral conditioning at 2.5% of the gait cycle has differential modulation on the ipsilateral soleus H-reflex. This modulation was dependent on when the test stimulus to the ipsilateral soleus was delivered during the gait cycle (mid vs. late stance phase), suggesting that contralateral antagonist sensory information is regulated by the phase of gait cycle. Overall, more investigations into this complex reflex response in humans will be required to make advancements in the understanding of crossed-spinal communication during human gait.

Disclosures: **A.M. Phipps:** None. **K. Katano:** None. **M. Enyart:** None. **D.M. Koceja:** None.

Poster

724. Defensive Behavior and Aggression

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 724.01/VV22

Topic: F.02. Behavioral Neuroendocrinology

Support: Macao Science and Technology Development Fund, FDCT, 011/2104/A1

Title: Built for fighting: neuroendocrine and transcriptomic regulation of aggression in a fighter strain of the siamese fighting fish Betta splendens

Authors: *D. GONÇALVES[1], A. RAMOS[1], C. HUANG[2], S. M. Y. LEE[2];
[1]Univ. of St. Joseph, Macau, Macao; [2]Univ. of Macau, Macao, Macao

Abstract: Across southern Asia, breeders have been selecting males of the Siamese fighting fish *Betta splendens* for fighting contests for a period of over 200 years. This strong directional selection has produced a variety of *B. splendens* morphologically different and more aggressive than wildtypes. Because wildtypes are still available in remote regions of southern Asia, the comparison with fighter strains provides an excellent opportunity to identify the genetic and neuroendocrine mechanisms underlying the expression of aggression in fish. In this study we compared the behavior, endocrine response and brain transcriptome profile of a strain of fighters with wildtypes when exposed to an aggressive challenge. As predicted, males fighters displayed more aggressive behavior both towards their mirror image and towards an opponent than wildtypes. Interestingly, female fighters were also more aggressive than female wildtypes, in spite of only males being the target of selection. In control animals (not displaying aggression), plasma androgen levels of 11-ketotestosterone (11KT), but not of testosterone (T), were more elevated in fighter males. Both fighter and wildtype males increased very significantly the secretion of both androgens in response to a 1h aggressive challenge. Whole brain gene expression profiling of wildtype and fighter males generated 74.7Gb of sequencing data that was assembled with Trinity, with 77% of unigenes annotated into known proteins. The analysis revealed significant differences in the brain transcriptome between wildtypes and fighters, both in the control and aggression-elicited treatment, with over 12,000 transcripts being differentially expressed, suggesting that selection for aggression has resulted in a highly divergent brain transcriptome profile. Exposure to the mirror treatment induced a similar number of differentially expressed genes in the two strains (443 in fighters and 312 in wildtypes), and enrichments detected by pathway analysis included steroid hormone biosynthesis, estrogen signaling and AVP/AVT pathways. In addition to providing the first annotated transcriptome for Betta splendens and identifying potential genomic pathways relevant for the expression of aggressive behavior, the study shows that selection for aggression produced a significantly altered brain transcriptome in males and co-selected high aggression levels also in females. The results also suggest that 11KT may be a key hormone modulating the high levels of aggression observed in males of the fighting strain.

Disclosures: D. gonçalves: None. **A. Ramos:** None. **C. Huang:** None. **S.M.Y. Lee:** None.

Poster

724. Defensive Behavior and Aggression

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 724.02/VV23

Topic: F.02. Behavioral Neuroendocrinology

Support: NSF IOS 1256898

NSF IOS 1257679

Title: Investigating the role of serotonin receptor subtypes mediating aggressive behavior in a novel species

Authors: *A. BUBAK[1], M. J. WATT[2], K. J. RENNER[3], J. G. SWALLOW[4];
[1]Biol., Univ. of Colorado-Denver Anschutz Med. Campus, Denver, CO; [2]Ctr. for Brain and Behavior Research, Basic Biomed. Sci., [3]Biol., Univ. of South Dakota, Vermillion, SD;
[4]Integrative Biol., Univ. of Colorado-Denver, Denver, CO

Abstract: Aggression is a complex and nearly universal behavior, documented in animal species ranging from humans to flies. Based on comparative research investigating the proximate mechanisms responsible for the appropriate perception and execution of aggressive behaviors, it is clear that genes and neurochemicals (i.e., biogenic amines, hormones, neuropeptides) that mediate this suite of behaviors are conserved across a wide range of taxa. The serotonergic (5-HT) system, in particular, shares deep evolutionary origins between vertebrates and invertebrates and has been largely implicated in aggressive behavior. However, most of our understanding with respect to the mechanisms of the serotonergic system derive from manipulations that result in global 5-HT changes. To better understand the system and the role of 5-HT we need to target individual genes such as receptor subtypes. Additionally, a large majority of this work has focused on a few model organisms, predominantly rodents and fruit flies, with the primary focus on males. This has led to significant gaps in our understanding of the conserved mechanisms that govern aggressive behavior as well as the substantial differences between sexes. We use a novel species, the stalk-eyed fly *Teleopsis dalmanni*, to explore the conserved underlying genetic and neurochemical mechanisms that mediate aggression. Members of this species are sexually dimorphic; males have exaggerated eyestalks that protrude laterally from their head, which they use as aggressive signals and weapons against conspecifics. Females are relatively docile compared to males, rarely engaging in aggressive conflicts with other females. Using next generation sequencing (RNA-seq) and RNA interference (siRNA), we are able to uncover the serotonergic-mediated mechanisms responsible for the sexually dimorphic aggressive behavioral response in a novel species.

Disclosures: **A. Bubak:** None. **M.J. Watt:** None. **K.J. Renner:** None. **J.G. Swallow:** None.

Poster

724. Defensive Behavior and Aggression

Location: Halls B-H

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Program#/Poster#: 724.03/VV24

Topic: F.02. Behavioral Neuroendocrinology

Support: NIH Grant DA10547

Title: 5HT3 receptor blockade during AAS withdrawal reduces AAS-enhanced anxiety without altering aggression

Authors: *T. R. MORRISON, L. RICCI, R. H. MELLONI, Jr;
Psychology, Northeastern Univ., Boston, MA

Abstract: Despite legal prohibitions against anabolic/androgenic steroids (AAS) in the US, usage rates amongst high school aged males are steadily rising and range between 2-5%, with some states reporting abuse rates as high as 8%. The AAS use during adolescence is associated with a host of risk factors and traits that include increased violence and aggressive behavior. Alongside aggression, AAS exposure during adolescence is also linked to an increased incidence of anxiety disorder diagnoses during adulthood that occur after the cessation of AAS abuse. Relatedly, adolescent males with *low* circulating testosterone report higher levels of anxiety, while those with *high* testosterone show no changes in anxiety, but exhibit *high* levels of aggression. Together, these data suggest that androgen fluctuations inversely affect aggression and anxiety, perhaps indicating that substrates of these behaviors are linked. Data generated in our lab support this hypothesis and show that AAS exposure during adolescence increases aggression and reduces anxiety. During withdrawal from AAS, anxiety levels rise significantly in the presence of normalized aggression. The effects of AAS on aggression and anxiety are likely regulated by 5HT and AVP signaling interactions in the lateral anterior hypothalamus (LAH) since, for example, AAS-aggression levels positively correlate with AVP fiber density in the presence of lower anxiety and reduced 5HT afferents in the LAH. Conversely, during withdrawal when anxiety levels are high, 5HT signaling remains reduced while AVP fibers and aggressive behavior return to control levels. Recently we showed that 5HT agonist injection in the LAH increases anxiety during AAS exposure. Along with previous reports showing that 5HT3 agonists increase aggressive behavior, 5HT3-activated anxiety suggests that this receptor is an important modulator of the relationship between aggression and anxiety after AAS exposure. To build on this notion, we measured aggression and anxiety during AAS withdrawal after injection of 5HT3 antagonists in the LAH. Blockade of 5HT3 receptors reduced AAS-withdrawal induced anxiety without affecting aggression. These data suggest that 5HT3 receptors regulate the anxiety component of the LAH circuit, and may indicate that the LAH modulates both

aggression and anxiety through a single upstream AVP pathway that diverges into parallel downstream mechanisms regulated by different populations of 5HT receptors.

Disclosures: **T.R. Morrison:** None. **L. Ricci:** None. **R.H. Melloni:** None.

Poster

724. Defensive Behavior and Aggression

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 724.04/VV25

Topic: F.02. Behavioral Neuroendocrinology

Support: ZIA-MH-002498-24

Title: Acute manipulation of Avpr1b CA2 neurons regulates expression of maternal aggression in mice.

Authors: ***S. WILLIAMS**[1], H.-J. LEE[2], J. SONG[3], W. YOUNG[3];
[1]Section on Neural Gene Expression, NIMH, Bethesda, MD; [2]Kyungpook Natl. Univ. Sch. of Dent., Daegu,, Korea, Republic of; [3]Natl. Inst. of Mental Hlth., Bethesda, MD

Abstract: Avp activation of the Avpr1b subtype is necessary for social recognition and aggression in both males and females. Avpr1b expression in the central nervous system is strictly limited to hippocampal CA2 neurons. We've shown that replacement of Avpr1b into the CA2 of Avpr1b knockout (KO) males via viral (pan-neuronal) expression rescued the behavioral deficits in aggression. This study indicated that activity of Avpr1b in the CA2 during adulthood, but not development, was important for regulating resident-intruder aggression. We are now interested in determining the causal role of Avpr1b neuronal activity in aggressive behavior. The use of designer receptor exclusively activated by designer drugs (DREADDs) allows for non-invasive acute manipulation of neuronal excitability in a cre-dependent manner. We created a transgenic mouse line that 'knocked-in' Cre-recombinase under the control of the Avpr1b promotor (Avpr1b-Cre), such that the Avpr1b was replaced by the Cre gene. Double in situ hybridization confirmed Cre expression in Avpr1b CA2 neurons in heterozygous adult mice. This line of mice was used to test two hypotheses: 1) Acute enhanced excitability will rescue deficits observed in Avpr1b KO female mice; and 2) acute inhibition will reduce female aggression. Experiment 1: Avpr1b-Cre heterozygous and Avpr1b-Cre KO female mice were injected with a Cre-dependent virus expressing either DREADD-Gq or control fluorescent marker. Experiment 2: Avpr1b-Cre heterozygous females were injected with a Cre-dependent DREADD-Gi or control fluorescent marker. Females were then paired with males until pregnancy was confirmed, and the male was removed. Females were administered CNO (ligand for DREADD) prior to resident-intruder

aggression testing. Tests were repeated several times across the mid-lactational period. Behavior was scored for latency and duration of aggression. Pup retrieval was recorded following aggression tests. Preliminary results suggest that Avpr1b-Cre KO mice injected with DREADD-Gq are more likely to attack and show a shorter latency to attack compared to KOs injected with GFP. Furthermore, Avpr1b-Cre mice injected with DREADD-Gi show increased latency to attack ($p < 0.05$) and decreased duration of aggression ($p < 0.05$) compared to Avpr1b-Cre mice injected with GFP. Manipulation of CA2 neuronal activity does not appear to affect maternal care.These data suggest that the acute activity of CA2 neurons is important for the expression of typical maternal aggression. These data add to our understanding the precise temporal time course of CA2 neuronal activity in regulation aggressive behaviors and indicates that effects are not sex-specific.

Disclosures: **S. Williams:** None. **H. Lee:** None. **J. Song:** None. **W. Young:** None.

Poster

724. Defensive Behavior and Aggression

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 724.05/DP08 (Dynamic Poster)

Topic: F.02. Behavioral Neuroendocrinology

Title: The role of the ventromedial hypothalamus during confrontation against conspecific aggressors

Authors: *L. WANG, D. LIN;
New York Univ. Sch. of Med., New York, NY

Abstract: In the wild, male animals fight for limited resources like food, mates, and territory. When they fight, they chase, lunge, and bite other males. After several rounds of confrontations, the more aggressive animals win the fight, and the losers hold up front paws in front of their chest to protect their core and actively defend themselves. These attack and defense scenarios can also be observed in the laboratory by using mice as an animal model. Previous studies in mice, rats, and hamsters have shown that c-fos, an immediate early gene, was elevated in the ventromedial hypothalamus (VMH) in males that were defeated by other conspecific aggressors (Kollack-Walker et al., 1997; Motta et al., 2009; Silva et al., 2013). The aim of our study is to understand the role of the VMH during confrontation against aggressive males. We first examined the expression patterns of c-fos after the residents animals were attacked by an aggressor introduced into their home cages. Consistent with previous findings, we observed elevated c-fos in the VMH, suggesting the involvement of the cells during confrontation against aggressors (Kollack-Walker et al., 1997; Motta et al., 2009; Silva et al., 2013). To further

understand the behavioral events, during which the VMH cells are activated, we performed electrophysiological recordings in VMH in freely moving animals. We observed acute increase of the VMH cell activity as the animal got attacked by the aggressor but not during his social investigation of the aggressor, indicating that the role of cells may be related to the physical defensive action of the animals. To test this hypothesis, we then optogenetically activated the VMH cells and observed light-bounded defensive behaviors, such as immobility, upright postures, and dashing. Taken together, our results suggest that the VMH cells may play an essential role in conspecific defense.

References: Kollack-Walker, S., S. J. Watson, and H. Akil, 1997, Social stress in hamsters: defeat activates specific neurocircuits within the brain: J Neurosci, v. 17, p. 8842-55. Motta, S. C., M. Goto, F. V. Gouveia, M. V. Baldo, N. S. Canteras, and L. W. Swanson, 2009, Dissecting the brain's fear system reveals the hypothalamus is critical for responding in subordinate conspecific intruders: Proc Natl Acad Sci U S A, v. 106, p. 4870-5.
Silva, B. A., C. Mattucci, P. Krzywkowski, E. Murana, A. Illarionova, V. Grinevich, N. S. Canteras, D. Ragozzino, and C. T. Gross, 2013, Independent hypothalamic circuits for social and predator fear: Nat Neurosci, v. 16, p. 1731-3.

Disclosures: L. Wang: None. **D. Lin:** None.

Poster

724. Defensive Behavior and Aggression

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Topic: F.02. Behavioral Neuroendocrinology

Support: Smoking Research Foundation

JSPS KAKENHI 25670121

Title: TRPA1 is indispensable to escaping from predator

Authors: R. AKAHOSHI[1], Y. KATAYAMA[1], R. YAMAGUCHI[2], T. YONEMITSU[3], J. L. PAULI[2], H. KASHIWADANI[2], *T. KUWAKI[2];
[1]Dept. of Physiol., Kagoshima Univ. Sch. of Med., Kagoshima, Japan; [2]Kagoshima Univ-Grad Sch. Med., Kagoshima, Japan; [3]Anesthesiol., Fujimoto Gen. Hosp., Miyakonojo, Japan

Abstract: Transient receptor potential ankyrin 1 (TRPA1), a member of the TRP superfamily, is expressed in a subset of sensory neurons and mediates nociception evoked by pungent chemicals. We have reported that TRPA1 knockout mice (KO) failed to avoid entering a chamber filled

with vapor of formalin, allyl isothiocyanate, and acrolein and proposed that TRPA1 serves as a frontline sensor to avoid environmental chemical threats (Sci Rep 3: 3100, 2013). In this study, we tested our hypothesis that TRPA1 also play a role in escaping from predator. As expected, TRPA1-KO failed to avoid fox feces and 2,4,5-trimethylthiazoline (TMT), a synthetic predator odor isolated from fox anal secretions. TRPA1-KO also failed to increase blood ACTH when exposed to TMT, but normally found buried food pellet indicating normal olfaction at least to smell of food. Nasal administration of AP-18, a specific blocker to TRPA1, in the wild-type mice reproduced the defect in TRPA1-KO. Finally, immunohistochemical examination revealed that TRPA1 is expressed in the nerve bundles in the respiratory turbinates of the nasal cavity. Taken together, we conclude that TRPA1 is indispensable to escaping from predator and that pungency of TMT, in addition to its smell, is important factor as a threatening cue.

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Poster

724. Defensive Behavior and Aggression

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Program#/Poster#: 724.07/WW1

Topic: F.02. Behavioral Neuroendocrinology

Support: NSF 1355163

Title: Oxytocin decreases aggression during courtship and increases investigation of intruders in the California mouse

Authors: *C. D. GUOYNES, C. MARLER;
Psychology, UW Madison, Madison, WI

Abstract: The neuropeptide hormone oxytocin (OT) plays a significant role in social bonding, social recognition, and anxiolytic behavior in both human and animal models. We examined the acute effects of intranasal OT (0.8 IU/kg) in male California mouse (*Peromyscus californicus*), a monogamous rodent that forms reliable pair bonds for mates and exhibits aggression towards territorial intruders. Previous research in prairie voles has shown that an acute dose of OT can decrease latency to form a partner preference; however, it is also important to ask how oxytocin influences behavioral interactions both before and after pair bond formation has occurred. Our goal was to determine the role of OT in males during critical social periods: pair bond formation, intrasexual aggression after pair bonding, pup retrievals with first litter, and partner preference after weaning of first litter. Males were treated with 25 uL of OT or saline 5 minutes before each

behavioral test, receiving a total of four treatments of either OT or saline (N=12 per group). In the first 10 minutes of pairing, the OT group engaged in significantly fewer aggressive interactions than the saline group (t=2.19, p=0.04). During a same-sex intruder encounter in their home territory, males given oxytocin showed significantly greater anogenital sniffing (t=2.3, p=0.03) with no change in number of aggressive attacks. Results from other behavioral tests will be reported. Based on the hypotheses that OT increases aggression towards the out-group, but increases affiliation towards the in-group, we hypothesize that OT will increase a number of behavioral traits: responsiveness to pup calls and pup licking and grooming during pup retrievals, allogrooming towards partner and aggression towards stranger in the partner preference tests. In summary, OT administered to males has different behavioral effects depending on the social context and appears to influence a variety of aggressive and affiliative behaviors, including during pair bond initiation in male California mice. These results are similar to the effects seen in male prairie voles with the exception that vasopressin—not oxytocin—is implicated in pair bond initiation for male voles.

Disclosures: C.D. Guoynes: None. **C. Marler:** None.

Poster

724. Defensive Behavior and Aggression

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Topic: F.02. Behavioral Neuroendocrinology

Support: IOS 1256898

Center for Brain and Behavioral Research pilot grant, USD

Title: Aggressive interactions between mantis shrimp (Neogonodactylus oerstedii) are mediated by brain monoamine levels and recognition of previous opponents

Authors: A. D. KOCH[1], J. D. W. YAEGER[1], M. W. BUCHANAN[1], M. L. PORTER[2], M. J. WATT[3], *K. J. RENNER[1];
[1]Biol., Univ. of South Dakota, Vermillion, SD; [2]Biol., Univ. of Hawaii at Manoa, Honolulu, HI; [3]Basic Biomed. Sci., Sanford Sch. of Med., Vermillion, SD

Abstract: The mantis shrimp *Neogonodactylus oerstedii* is a solitary marine crustacean that actively defends territory from conspecifics. Serotonin (5-HT) has been linked with aggression in several crustacean species based on exogenous 5-HT application. However, whether endogenous monoamines in the crustacean brain mediate aggression and contest outcome is relatively

unknown. In other species, contest outcome is also influenced by recognition of previously-fought opponents, as it can prevent further costly disputes when the likely outcome is already known. Some species of mantis shrimp exhibit brief memory of previous opponents as indicated by olfactory-based avoidance, but is not known if this alters subsequent aggression. Here, we first staged fights between size- and sex-matched pairs of *N. oerstedii* and measured brain octopamine, dopamine (DA) and 5-HT immediately afterwards, and show that contest losers have higher levels of 5-HT and DA relative to winners. Next, we determined if opponent recognition would reduce the duration and intensity of aggressive interactions between previously-fought individuals. Size and sex matched *N. oerstedii* engaged in two interactions with the same opponent but separated by a 24 h period, with control groups comprising winners and losers from the first fight paired with size-matched novel opponents of unknown status in the second contest. Shrimp paired with familiar opponents exhibited decreases in interaction length, number of strikes and threat displays in the second fight, with winning and losing status remaining consistent across contests. In contrast, individuals confronted with an unfamiliar opponent maintained their previous level of aggression, and 23% of subjects were able to reverse prior contest outcome. Our results suggest endogenous brain DA and 5-HT mediate initial expression of aggression in *N. oerstedii*, and that contest outcome is also heavily influenced by memory of previous opponents for at least 24 h after social status has been established. We are now testing if pretreatment with exogenous 5HT or DA can reverse the outcome of the second fight in familiar *N. oerstedii* opponents, which will provide insight into proximate mechanisms underlying formation and maintenance of social hierarchies.

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Poster

724. Defensive Behavior and Aggression

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Program#/Poster#: 724.09/WW3

Topic: F.02. Behavioral Neuroendocrinology

Support: ERC Advanced Grant

Title: Stereological analysis of intact ventromedial hypothalamus morphology and synaptic organization: new tools for understanding juvenile social behaviour plasticity

Authors: *A. CICCARELLI, R. PAOLICELLI, D. WEIJERS, F. ZONFRILLO, E. PERLAS, G. BOLASCO, C. GROSS;
Mouse Biol. Unit, EMBL, Monterotondo, Italy

Abstract: Changes in social behaviour induced by juvenile conspecific interactions is a critical and pro-survival form of behavioral plasticity conserved across species. This form of neuroadaptation is supposed to depend on the high degree of brain plasticity present during development; however it is still unclear if a critical period for shaping social behaviour exists and from which brain region it is regulated. Ventromedial hypothalamus (VMH) has a pivotal role in regulating many types of social behaviours, such as defensive behaviour, aggression and sexual receptivity. We hypothesize that in mice structural plasticity occurring in this hypothalamic brain region could underlie behavioural changes induced by stressful social experiences during development. In this study we developed a behavioural protocol aimed to study the effect of repetitive social defeat on juvenile mice (from p22 to p31). Preliminary data show that juvenile-defeated mice display an increase in adult aggressive behaviour and social avoidance indicating a long-lasting form of behavioural plasticity. To study structural plasticity in VMH we first performed a detailed characterization of VMH synaptic network in control mice. We used a transgenic mouse line (*Nr5a1::*Cre; *Rosa-CAG::*LSL-Synaptophysin-GFP; *Rosa-CAG*::LSL-Tomato) that allows us to visualize: a) the majority of VMH neurons and their axonal/dendritic morphology; 2) the VMH excitatory synapses (presynaptic terminals) within the VMH (excitatory collaterals) and in VMH target regions (e.g., PAG). Quantitative analysis using confocal microscopy and immunofluorescence revealed that VMH collateral synapses represent approximately 30% of total VMH inputs and are denser in the rostral part of VMH. Double staining with presynaptic markers revealed that VMH collaterals express mainly Vglut2 and this region is enriched as well in synaptic inputs arising from extra-VMH excitatory neurons (Vglut1-positive). Next, in order to systematically describe the entire VMH morphology and collateral synapses, we clarified the whole VMH of adult transgenic mice using a modified CLARITY protocol. With lightsheet microscopy we visualized the intact VMH and analysed the full population of VMH Nr5a1-positive neurons and the excitatory collateral synapses. Automatic quantification revealed that the VMH total number of neurons is approximately 32000 and 3D rendering showed new morphological properties of this region. In conclusion, here we describe a new form of juvenile-induced social behavioural plasticity and provide new tools for systematic analysis of intact VMH and its structural plasticity in mice.

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Poster

724. Defensive Behavior and Aggression

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Topic: F.02. Behavioral Neuroendocrinology

Support: Supported by the Military Operational Medicine Research Program, US Army

Title: Subacute neuroendocrine and behavioral effects of underwater trauma and predator exposure in rats

Authors: K. D. CRAVEDI[1], C. V. VUONG[2], J. C. SOUSA[2], S. R. MARCSISIN[2], *N. L. MOORE[3];
[1]Ctr. for Military Psychiatry and Neuroscience, Military Psychiatry Br., [2]Div. of Exptl. Therapeutics, Drug Metabolism and Disposition, [3]Ctr. for Military Psychiatry and Neuroscience, Behavioral Biol. Br., Walter Reed Army Inst. of Res., Silver Spring, MD

Abstract: The physiological response in rats to traumatic stress can be acutely and chronically measured using a multitude of neuroendocrine and behavioral markers. Responses of these interrelated neuroendocrine and behavioral markers may vary from molecule to molecule and behavior to behavior, and outside factors like the type of stressor or the phase in circadian cycle may also influence observed changes. While rodent models of traumatic stress have provided a glimpse into the physiological response to acute and chronic stressors, few studies have examined a more comprehensive interrelated molecular and behavioral approach. The aim of the present study is to provide a more complete examination to describe the subacute effects of traumatic stress exposure and recovery to a panel of neuroendocrine markers, and to compare those alongside acute and long lasting behavioral responses such as exploratory behavior and acoustic startle response, over the circadian cycle. Rats were exposed to either underwater trauma (UWT) or predator exposure (PRED). Then repeated behavioral tests (exploratory behavior on the elevated plus maze, acoustic startle) were conducted across 5 individual time points over a 7 day period. Additionally, blood samples were simultaneously collected over 10 time points across the circadian cycle in yoked stress-exposed cohorts. Neuroendocrine markers were quantified from serum using LC-MS or ELISA, analyzed over time, and compared against behavioral responses at corresponding time points. Preliminary data show differential responses for UWT and PRED across the experimental timeline, and distinct response timelines for the different measures collected. Supported by the Military Operational Medicine Research Program, US Army Medical Research and Materiel Command. Material has been reviewed by the Walter Reed Army Institute of Research. There is no objection to its presentation and/or publication. The opinions or assertions contained herein are the private views of the author, and are not to be construed as official, or as reflecting true views of the Department of the Army or the Department of Defense. Research was conducted in compliance with the Animal Welfare Act and other Federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, NRC Publication, 2011 edition. All procedures were reviewed and approved by the WRAIR Institutional Animal Care and Use Committee, and performed in facilities accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International.

Disclosures: K.D. Cravedi: None. **C.V. Vuong:** None. **J.C. Sousa:** None. **S.R. Marcsisin:** None. **N.L. Moore:** None.

Poster

724. Defensive Behavior and Aggression

Location: Halls B-H

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Program#/Poster#: 724.11/WW5

Topic: F.02. Behavioral Neuroendocrinology

Title: Seasonal variations in the dorsolateral and medial cortex, the reptilian homologue of the hippocampus

Authors: A. D. ABDILAHI[1], A. B. DAY[1], *R. E. COHEN[2];
[1]Minnesota State University, Mankato, Mankato, MN; [2]Biol. Sci., Minnesota State Univ. Mankato Dept. of Biol. Sci., Mankato, MN

Abstract: The hippocampus is a region of the brain involved in spatial learning and memory and is a site of neural plasticity in the adult brain. Specifically, this region has been shown to add new neurons in adulthood in a variety of animals, as well as have altered morphology (volume) under different seasonal conditions in some bird species. In the seasonally breeding green anole lizard, *Anolis carolinensis*, breeding season (BS) lizards exhibit territorial and aggressive behaviors to vigorously compete for large territories, with a decrease in these behaviors and territory sizes during the non-breeding season (NBS). Previous work has shown that morphology of brain areas involved in reproduction is altered seasonally and this is likely due to seasonal changes in steroid hormones, specifically testosterone (T) and its metabolites, estradiol (E2) and 5α-dihydrotestosterone (DHT). It is currently unknown whether there are seasonal changes to the anole hippocampus. To investigate potential seasonal effects in the lizard homologue of the hippocampus, the dorsolateral cortex (DC) and medial cortex (MC), we examined 1) morphology in BS and NBS males and 2) neuron addition in BS males treated with various hormones. In experiment 1, we obtained males during the BS and NBS, collected brains, and examined volumes in Nissl stained sections. In experiment 2, we gonadectomized BS males and implanted subcutaneous capsules containing T, E2, DHT, or left empty (blank). After hormone implantation, animals were injected with bromodeoxyuridine (BrdU; 50mg/kg) once per day for three days and brains collected after 25 days. Immunohistochemistry for BrdU and HuC/D (a neuronal marker) was performed to determine the number of new neurons present in the DC after treatment. Preliminary results for experiment 1 have shown no significant difference in DC and MC volume between BS and NBS lizards ($t < 1.10$, $p > 0.385$, $n = 2$). Additionally, experiment 2 preliminary results suggest that there may be a decrease in new neuron survival with T ($n = 5$) compared to blank ($n = 3$; $t = 2.35$, $p = 0.057$). These preliminary results suggest that DC and MC volume does not change seasonally, but steroid hormones may promote increased turnover of new neurons. Data collection for both experiment 1 and 2 is in progress and we will also examine new neuron numbers in animals treated with E2 and DHT.

Disclosures: **A.D. Abdilahi:** None. **A.B. Day:** None. **R.E. Cohen:** None.

Poster

724. Defensive Behavior and Aggression

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 724.12/WW6

Topic: F.02. Behavioral Neuroendocrinology

Support: NIH Grant 1R01MH104603-01A1

KU SIG INS0072533

Title: Gene x environment interactions in pathological aggression modify the expression and function of CB1 receptors: relevance to the comorbidity between antisocial behavior and cannabis use

Authors: *K. M. MCFARLIN[1,2], M. BORTOLATO[2];
[1]Univ. of Kansas, Lawrence, KS; [2]Dept. of Pharmacol. and Toxicology, Univ. of Utah, Salt Lake City, UT

Abstract: Antisocial behavior (AB) is a neuropsychiatric disorder characterized by inappropriate and often exaggerated aggressive and violent responses. Ample evidence has shown that the pathogenesis of aggression reflects the synergism of genetic and environmental vulnerability factors; in particular, the best-characterized gene x environment (GxE) interaction in AB concerns: 1) a low-activity variant of the *MAOA* gene, encoding the enzyme monoamine oxidase A; and 2) child neglect and/or abuse. Our lab has recently developed the first mouse model of this GxE interaction, based on exposing hypomorphic MAOA mutants ($MAOA^{Neo}$) to early stress (ES) during the first week of postnatal life. In line with clinical evidence, adult male $MAOA^{Neo}$ mice subjected to this manipulation exhibit a marked increase in aggression, as compared to their non-stressed (NS) or wild-type (WT) controls. One of the major problems in AB management lies in its association with abuse of psychoactive substances, and, most notably, cannabis. This comorbidity may reflect a self-therapeutic attempt; indeed, low doses of the main psychoactive ingredient of cannabis, Δ^9-tetrahydrocannabinol (THC), have been shown to reduce aggression. The antiaggressive effects of cannabis are posited to be mediated by cannabinoid 1 receptors (CB1Rs) in key brain regions that orchestrate the emotional response to threat, such as amygdala and hypothalamus. Nevertheless, the neurobiological bases of the comorbidity between cannabis abuse and aggression remain unclear. To address this issue, we hypothesized that the aggression in ES-exposed $MAOA^{Neo}$ mice may be underpinned by brain-regional changes in CB1Rs. In line with this idea, we found that adult male ES-$MAOA^{Neo}$ mice displayed

increased levels of hypothalamic CB1Rs. Furthermore, low-dose THC (0.03 mg/kg, IP) selectively ablated aggressive responses in adolescent ES-exposed $MAOA^{Neo}$ mice, without affecting their locomotor behavior. Notably, these effects were dissociated from changes in anxiety-related changes and prevented by a selective CB1R antagonist, AM251 (1 mg/kg, IP). These results collectively suggest that CB1Rs are directly and selectively implicated in the regulation of GxE interactions in AB. Ongoing studies in our lab are currently assessing the temporal trajectory of the alterations in the endocannabinoid system with respect to the ontogeny of aggression, as well as the anti-aggressive mechanisms of THC.

Disclosures: K.M. McFarlin: None. **M. Bortolato:** None.

Poster

724. Defensive Behavior and Aggression

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 724.13/WW7

Topic: F.02. Behavioral Neuroendocrinology

Title: The 5α-reductase inhibitor Finasteride increases suicide-related aggressive behaviors in a schizophrenia-like phenotype

Authors: *C. MAURICE-GÉLINAS[1], C. MONPAYS[1], J. DESLAURIERS[2], P. SARRET[1], S. GRIGNON[1,3];
[1]Univ. De Sherbrooke, Sherbrooke, QC, Canada; [2]Univ. of California, San Diego, CA; [3]Dept. of Psychiatry, Ctr. Hospitalier Universitaire de Sherbrooke, Sherbrooke, QC, Canada

Abstract: Death by suicide is 4 to 10 times higher among schizophrenia patients than in the general population. At odds with the general population, there is now compelling evidence suggesting that the pathophysiology of suicide in schizophrenia does not involve serotonergic neurotransmission. We recently described and characterized a murine Two-Hit Model of Suicide-related behaviors in a schizophrenia-like context (THMS) involving gestational inflammation followed by adolescent social isolation (SI). In this model, pregnant C57BL/6 mice are injected with polyinosinic/polycytidylic acid (PolyI:C) 20 mg/kg at gestational day 12 and their offspring are submitted to SI from postnatal (PN) days 21 to 53. In this THMS, clozapine normalized prepulse inhibition, aggressiveness, impulsivity and anxiety-like behaviours (1). While the basis of clozapine superior effectiveness on suicidal behaviors in schizophrenic patients is not well understood, previous works has revealed that clozapine alters central neurosteroid (NS) levels, such as allopregnanolone.
Methods. As a preliminary step to ascertain a potential NS involvement, we submitted control and THMS mice to daily intraperitoneal administration (from PN43 to PN53) of 5-alpha-

reductase inhibitor finasteride (50 mg/kg) before clozapine (3 mg/kg) or vehicle injection. Clozapine and finasteride were administered 30 min and 20 h before behavioural testing, respectively. Prepulse inhibition (PN50), elevated plus maze (PN51), resident intruder test (PN52) and forced swim test (PN53) were then performed to assess the sensorimotor gating, anxiety-like behaviours, aggressiveness, and depressive-like responses, respectively. Mice were then sacrificed for biochemical analyses.
Results. As previously reported, male THMS mice were more aggressive and impulsive than controls. In the THMS only, finasteride treatment further increased aggressiveness (# attacks +123 % ($p<0.01$); time spent attacking +70% ($p<0.05$)) and impulsiveness (55 % decrease in the latency before the first attack, $p<0.05$). Clozapine essentially normalized these parameters under both conditions (THMS or THMS + finasteride), without any evidence of a specific interaction with finasteride.
Conclusion. These results thus suggest that neurosteroids may intervene in the control of aggression in the THMS model, but do not specifically underlie the beneficial effects of clozapine.
(1) Deslauriers, J., et al., *A two-hit model of suicide-trait-related behaviors in the context of a schizophrenia-like phenotype: Distinct effects of lithium chloride and clozapine.* Physiol Behav, 2016. **156**: p. 48-58.

Disclosures: C. Maurice-Gélinas: None. **C. Monpays:** None. **J. Deslauriers:** None. **P. Sarret:** None. **S. Grignon:** None.

Poster

724. Defensive Behavior and Aggression

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 724.14/WW8

Topic: F.02. Behavioral Neuroendocrinology

Support: FAPESP

CNPq

Title: Evaluation of aggressive behavior in an animal model of perimenopause

Authors: *L. C. DALPOGETO[1], N. PESTANA-OLIVEIRA[2], J. A. ANSELMO-FRANCI[3], C. M. LEITE[3];
[1]Univ. De Sao Paulo, Ribeirao Preto, Brazil; [2]Dept. de Fisiologia, FMRP-USP, [3]Dept. de Morfologia, Fisiologia e Patologia Básica, FORP-USP, Univ. de São Paulo, Ribeirao Preto, Brazil

Abstract: During the perimenopause - a transition period that precedes menopause - women experience mood disorders like anxiety, depression as well as episodes of irritability and aggressiveness, which can be related to the hormonal changes like low levels of progesterone and testosterone as well as elevated or normal concentrations of estrogens. In rodents, 4-vinylcyclohexene diepoxide (VCD) induces a gradual depletion of ovarian follicles, modeling the transition to menopause in women. We aimed here at investigating if aggressive behavior is also expressed in this animal model. Female Wistar rats (28 days old) were treated with VCD (160mg/Kg s.c) or oil for 15 consecutive days. Sixty five days after the onset of VCD treatment, rats were placed in individual cages, and the estrous cycle was verified daily. Around 10 days after, rats with regular cycles in the diestrus phase were submitted to social instigation for 5 min followed by the Resident-Intruder test for 10 min. The recorded behavior was analyzed for offensive aggression (lateral threat, clinch attack, keep down, bites, upright posture, chase and move towards) and defensive aggression (move away, defensive upright and submissive posture). VCD-treated rats exhibited higher frequency of offensive aggression, lower frequency of defensive aggression and lower attack latency ($p<0,05$). There was a significant increase in the offensive behaviors clinch attack and bites and a decrease in the defensive behavior move towards ($p<0,05$). There was no difference among non-aggressive behaviors like social and non-social exploration, rearing and grooming, suggesting that locomotor activity was not impaired by the VCD-treatment. The higher aggressive behaviors displayed by rats in periestropause are similar to those exhibited by women, indicating that this animal model is suitable for the studies of perimenopause. These results incite new researches about the impact of the perimenopausal hormonal changes in the neural mechanisms that regulate aggressiveness.

Disclosures: L.C. Dalpogeto: None. **N. Pestana-Oliveira:** None. **J.A. Anselmo-Franci:** None. **C.M. Leite:** None.

Poster

724. Defensive Behavior and Aggression

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 724.15/WW9

Topic: F.02. Behavioral Neuroendocrinology

Support: Burroughs wellcome fund career award for medical scientists

Title: Developmental gating of aggressive behaviors in *Drosophila*

Authors: *E. H. MOSCATO, B. MAINWARING, M. S. KAYSER;
Dept. of Psychiatry, Univ. of Pennsylvania, Philadelphia, PA

Abstract: A critical challenge in neuroscience is determining how complex behaviors arise from rapidly changing circuitry in the brain. Early in life, animals initiate a broad repertoire of behaviors essential for survival, such as feeding, sleeping, and aggression. Somehow, young animals know how to engage in these activities, and for this reason, such innate behaviors are often referred to as "hard-wired". However, innate behaviors can arise from immature underlying neural processes, show dramatic ontogenetic change, and be altered by experience, indicating that these circuits are highly dynamic and modifiable. Aberrant emergence of social behaviors in early life is a hallmark of human neurodevelopmental disorders, emphasizing the importance in gaining a mechanistic understanding of behavioral ontogeny. We are using the model system *Drosophila* to dissect the neural, molecular, and genetic logic underpinning the gating of aggression in early life.
We have found that newly eclosed male flies do not exhibit aggressive behaviors for the first 24 hours of adult life; this finding does not reflect a generalized inability to enact complex behaviors, as male flies begin courting females as early as 4 hours post eclosion. As flies age, aggression becomes increasingly robust: older flies display a greater number of aggressive behaviors, a decreased latency to initiate aggression upon encountering another male fly, and increased tendency to establish dominance over a fighting partner. To discover novel regulators of aggression ontogeny, we have undertaken a thermogenetic screen to identify neurons and circuits that can drive aggression in juvenile flies. Activation of neurons defined by two Gal4 lines, YF1 and YF2, strongly promotes aggressive behaviors on the first day of adult life, when flies are normally non-aggressive. On-going work aims to determine how these neuronal populations interact with each other and with previously characterized regulators of mature aggression to modulate the developmental gating of aggressive behaviors in *Drosophila*. These studies will provide fundamental insights into the neurobiological mechanisms that regulate the maturation of essential behaviors.

Disclosures: **E.H. Moscato:** None. **B. Mainwaring:** None. **M.S. Kayser:** None.

Poster

724. Defensive Behavior and Aggression

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Topic: F.02. Behavioral Neuroendocrinology

Support: NIH Grant MH104603-01

NIH Fellowship F31NS093939

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NIH P20 GM103418

EU COST Action CM1103

Title: mGluR2 mediates the interaction between low MAO A activity and early stress model of aggression

Authors: *S. C. GODAR[1,2], L. J. MOSHER[1,2], C. M. JONES[1], H. STRATHMAN[1], S. SCHEGGI[3], C. GAMBARANA[3], M. DE MONTIS[3], M. BORTOLATO[2];
[1]Pharmacol. and Toxicology, Univ. of Kansas, Lawrence, KS; [2]Pharmacol. and Toxicology, Univ. of Utah, Salt Lake City, UT; [3]Mol. and Developmental Med., Univ. of Siena, Siena, Italy

Abstract: Pathological reactive aggression is a neurodevelopmental condition with devastating socioeconomic ramifications. The pathogenesis of this disorder is largely governed by the interplay between gene x environmental (GxE) factors. One of the best characterized genes for aggression is monoamine oxidase A (MAOA), the primary metabolic enzyme for serotonin (5-HT) and norepinephrine degradation. A host of evidence has shown that individuals with low MAOA activity allelic variants, who have been subjected to early life neglect and/or abuse, are predisposed to aggression and antisocial behaviors.
Based on these clinical findings, we developed a novel animal model of this GxE interaction by subjecting non-aggressive MAOA hypomorphic mutant mice ($MAOA^{Neo}$) to daily early stress (ES, maternal separation and saline injection) from postnatal day 1 through postnatal day 7 (PND1-7). Adult ES-$MAOA^{Neo}$ mice exhibit high levels of aggression compared to the wild-type littermates and non-stressed $MAOA^{Neo}$ counterparts. These behaviors were accompanied by a gradual reduction in N-methyl-D-aspartate receptor (NMDAR) expression and function in the prefrontal cortex in adulthood, likely contributing to a prefrontal disinhibition over subcortical circuits.
Previous studies revealed that ES-$MAOA^{Neo}$ pups displayed elevated levels of 5-HT, as well as the 5-HT receptor 2A (5-HT_{2A}) in the prefrontal cortex at PND7. Selective pharmacological 5-HT_{2A} blockade during the first postnatal week attenuated the aggressive behaviors and NMDAR deficits in ES-$MAOA^{Neo}$ mice, suggesting that 5-HT_{2A} hyperstimulation in early stages mediates aggression. However, it is unclear how NMDAR function in adulthood is affected by early 5-HT_{2A} receptor alterations.
Building on these premises, recent evidence has indicated that 5-HT_{2A} receptors inversely mediate the properties of metabotropic glutamate receptor 2 (mGluR2), a modulator of NMDAR function, through the formation of heteromeric complexes. Here we found that ES-$MAOA^{Neo}$ mice display reduced mGluR2 expression in the prefrontal cortex at PND 7. Pharmacological treatment with the mGluR2 positive allosteric modulator biphenylindanone A (BINA, 30 mg/kg, IP) during the first postnatal week elicited a robust reduction in aggression in ES-$MAOA^{Neo}$ animals. These findings suggest that the interaction between low MAOA activity and early stress in aggression is likely contributed by 5-HT_{2A} receptor-mediated disruption of mGluR2 during early stages.

Disclosures: **S.C. Godar:** None. **L.J. Mosher:** None. **C.M. Jones:** None. **H. Strathman:** None. **S. Scheggi:** None. **C. Gambarana:** None. **M. De Montis:** None. **M. Bortolato:** None.

Poster

725. Glucocorticoid Actions

Location: Halls B-H

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Program#/Poster#: 725.01/WW11

Topic: F.04. Stress and the Brain

Support: Pharmaceutical grant from Corcept Therapeutics awarded to MBS

Title: CORT 118335 and imipramine affect endocrine stress responses through different central mechanisms in male and female rats

Authors: *E. T. NGUYEN[1,3], J. L. CALDWELL[1], J. STREICHER[1], S. BERMAN[1], V. GHISAYS[1,2], C. M. ESTRADA[1,2], M. B. SOLOMON[1];
[1]Dept. of Psychiatry and Behavioral Neurosci., [2]Psychology Grad. Program, Univ. of Cincinnati, Cincinnati, OH; [3]Neurosci. Grad. Program, Univ. of Cincinnati Col. of Med., Cincinnati, OH

Abstract: Glucocorticoid dyshomeostasis is implicated in the pathophysiology of stress-related disorders (i.e., depression and anxiety). Glucocorticoids exert their biological effects by acting on Type I (mineralocorticoid MR) and Type II (glucocorticoid GR) receptors. Accordingly, compounds targeting both MR and GR are being pursued as putative antidepressants and anxiolytics. Women are twice as likely to suffer from stress-related disorders which may be due to sex differences in glucocorticoid production. For example, female rodents display heightened hypothalamic-pituitary-adrenal (HPA) axis activity (increased basal and stress-induced glucocorticoid concentrations) relative to male rodents. Previous data from our laboratory indicate that in most cases, compounds targeting GR dampen central (c-Fos), endocrine, and behavioral stress responses in males. Because of the dynamic interplay between MR and GR in regulating HPA axis tone, compounds targeting both receptor subtypes are of interest for stress-related pathology. In this vein, we have determined that CORT 118335, a dual selective GR modulator/MR antagonist, attenuates endocrine and metabolic stress responses in males. Given the female biased increase in endocrine and behavioral stress responses, this study sought to assess potential sex differences in the efficacy of CORT 118335 to modulate central (c-Fos), endocrine, and behavioral stress responses. To accomplish this goal, adult male and female rats were treated for 5 days with either vehicle, CORT 118335, or imipramine (tricyclic antidepressant) and exposed to restraint (Experiment 1) or forced swim test (FST) (Experiment 2) stress. CORT 118335 significantly dampened corticosterone stress responses in both sexes. Notably, imipramine dampened corticosterone stress responses in males, but increased corticosterone responses in females. The neuroendocrine stress dampening properties of CORT 118335 did not impact behavioral responses in the FST in either sex. CORT 118335 did not affect c-Fos expression in response to FST exposure in the hippocampus in males, but increased c-Fos expression in the CA1 in females. Interestingly, imipramine dampened c-Fos expression in

the hippocampus (CA1 and CA3) in males, but had no effect in these areas in females. Imipramine decreased c-Fos expression in the basolateral amygdala in both sexes, but increased c-Fos expression in the central amygdala in females only. These data indicate sex differences in the efficacy of these compounds to modulate central and endocrine stress responses and suggest differential recruitment of neural circuits during stress exposure in males and females.

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Poster

725. Glucocorticoid Actions

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 725.02/WW12

Topic: F.04. Stress and the Brain

Support: CONACYT 180009

DGAPA IN-204316

Title: Corticosterone interferes with the response of TRHergic hypophysiotropic neurons to neural stimulation by attenuation of CREB phosphorylation. Mechanism involves GR interaction with catalytic subunit of PKA.

Authors: *I. SOTELO RIVERA, R. M. URIBE, A. COTE-VÉLEZ, J.-L. CHARLI, P. JOSEPH-BRAVO;
Inst. de Biotecnologia, UNAM, Cuernavaca, Mexico

Abstract: Cold exposure activates the hypothalamic-pituitary-thyroid (HPT) axis, increasing the expression of thyrotrophin releasing hormone (TRH) in hypophysiotropic neurons of the paraventricular nuclei of the hypothalamus (PVN), TRH release into the portal blood, thyrotrophin (TSH) and thyroid hormone concentrations in serum. Stress inhibits the activity of the HPT axis and we have recently shown that a previous stress exposure as well as a corticosterone injection interferes with the response of the HPT axis to acute cold (1). We have characterized the response elements of the TRH gene promoter that bind phosphorylated CREB (CRE), and the dexamethasone activated glucocorticoid receptor (GR) GRE, and demonstrated,

in primary cultures of hypothalamic cells that the combined treatment of PKA activators and dexamethasone interfere with the stimulatory effect of cAMP on TRH mRNA levels, on TRH transcription and on pCREB binding to CRE or GR binding to GRE (2); the latter process does not involve chromatin compaction (I. Sotelo-Rivera in preparation). We now evaluated the degree of CREB phosphorylation in PVN-TRH neurons of rats injected with corticosterone and exposed 1h to cold, measuring the total amount of cells expressing proTRH mRNA (detected by in situ hybridization) and pCREB, measured by immunocytochemistry. pCREB and TRHergic/pCREB neurons were increased by cold exposure but this was attenuated by corticosterone treatment. We confirmed in neuroblastoma cell line SH-SY5Y the results previously obtained in hypothalamic primary cell culture providing clear evidence for diminished pCREB in cells coincubated with forskolin and dexamethasone compared to forskolin alone. This combined treatment attenuated the proper translocation of GR or PKAc to the nucleus, compared to that observed with cells incubated only with forskolin or dexamethasone. Coimmunoprecipitation analyses confirmed protein-protein interaction between GR:PKAc. These results strongly support that the interaction of GR with PKAc might be responsible for the diminished CREB phosphorylation induced by cAMP or neuronal activation.
(1) I. Sotelo-Rivera,*et al.*, 2014. *Journal of Neuroendocrinology,* 26, 861-869. (2) M. Y. Díaz-Gallardo et al., 2010. *Journal of Neuroendocrinology,* 22, 282-293.

Disclosures: I. Sotelo Rivera: None. **R.M. Uribe:** None. **A. Cote-Vélez:** None. **J. Charli:** None. **P. Joseph-Bravo:** None.

Poster

725. Glucocorticoid Actions

Location: Halls B-H

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Topic: F.04. Stress and the Brain

Support: 2R01 MH066958

IOS 1053716

Title: Membrane-initiated nuclear translocation of the glucocorticoid receptor in hypothalamic neurons

Authors: *J. R. RAINVILLE, N. VASUDEVAN, J. G. TASKER;
Cell & Mol. Biol., Tulane Univ., New Orleans, LA

Abstract: Classical glucocorticoid receptor (GR) signaling as a transcription factor begins with ligand binding to the intracellular GR, release of the GR from chaperone proteins and dimerization, and translocation of the GR dimer to the nucleus, where the GR binds to the glucocorticoid response element (GRE) to regulate GRE-dependent transcription. We studied GR nuclear translocation via activation of a putative membrane GR in a murine hypothalamic cell line, mHypoE-N42, which expresses endogenous GR. We found that the GR synthetic agonist dexamethasone (Dex) conjugated to bovine serum albumin (Dex-BSA), which is membrane limited, is able to rapidly induce nuclear translocation of the GR. Using a GR antibody and intensity analysis of the nuclear-to-cytoplasmic ratio of GR-immunoreactivity, we found that nuclear translocation of GR was increased equally by Dex and Dex-BSA within 20 minutes. We confirmed the Dex- and Dex-BSA-induced GR translocation to the nucleus in primary hypothalamic neurons cultured from male P1 C57/BL6 mice. The Dex-BSA-induced nuclear translocation suggests the mobilization of an unliganded GR by signaling from the membrane. Akt activation blocked Dex-BSA-, but not Dex-mediated GR nuclear translocation, suggesting an alternative nuclear localization pathway may be initiated by membrane signaling. COS7 cells, which have no endogenous GR, were transfected with GR-GFP, and responded to both Dex and Dex-BSA treatment with GR nuclear translocation, suggesting that GR is sufficient for membrane-initiated nuclear translocation of GR. Dex treatment resulted in an increase in GRE-mediated transcription, detected with a GRE-luciferase assay, but Dex-BSA treatment for up to an hour did not result in an increase in GRE-luciferase activity. This suggests that the mobilization of the unliganded GR by Dex-BSA does not produce a transcriptionally active GR at the GRE, and serves as a control for the restriction of the Dex-BSA actions to the membrane. Thus, signaling from an unidentified membrane-associated receptor stimulates nuclear trafficking of GR, which is not transcriptionally active at the GRE. However, the unliganded GR may act on other transcriptional regulators to mediate some of the genomic effects of glucocorticoids.

Disclosures: **J.R. Rainville:** None. **N. Vasudevan:** None. **J.G. Tasker:** None.

Poster

725. Glucocorticoid Actions

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Program#/Poster#: 725.04/WW14

Topic: F.04. Stress and the Brain

Support: NIH R01 MH066958.

Title: Intracellular signaling pathway of the membrane glucocorticoid receptor responsible for endocannabinoid modulation of excitatory synaptic inputs to hypothalamic neuroendocrine cells

Authors: *G. L. WEISS, C. HARRIS, S. DI, J. G. TASKER;
Tulane Univ., New Orleans, LA

Abstract: The hypothalamus controls homeostatic functions and acts as a site for crosstalk between brain circuits controlling fluid regulation, reproduction and the stress response. Glucocorticoids (GCs) secreted into the blood during stress activation of the hypothalamic-pituitary-adrenal (HPA) axis feed back on the hypothalamus to inhibit HPA activation and curtail the stress response. GCs have rapid inhibitory actions on neuroendocrine cells in the supraoptic and paraventricular nuclei (SON/PVN) by activation of a putative membrane glucocorticoid receptor and downstream synthesis of the endocannabinoid 2-arachidonoylglycerol (2-AG) and retrograde suppression of glutamate release at excitatory synapses. Here, we used whole-cell patch-clamp recordings and pharmacological manipulations in hypothalamic slices to further explore the intracellular signaling pathway responsible for 2-AG synthesis by activation of the membrane glucocorticoid receptor. Using the synthetic glucocorticoid dexamethasone (Dex), we found that the rapid glucocorticoid-induced 2-AG suppression of excitatory synaptic inputs to magnocellular neurons is dependent on the activation of PKA, PKC, ERK, and SRC signaling pathways, as well as on inositol triphosphate receptor-mediated calcium release. PKA was found to be upstream of PKC and ERK in the signaling pathway. Interestingly, while PKC inhibition blocked the glucocorticoid-induced endocannabinoid suppression of excitatory synaptic inputs, PKC activation alone was not sufficient to activate endocannabinoid release, which suggested a bifurcation in the intracellular signaling pathway. In support of this, phospholipase C activation stimulated the endocannabinoid-mediated suppression of excitatory inputs, suggesting that both phospholipase C-induced intracellular calcium release and PKC activation may be necessary for the endocannabinoid suppression of glutamate release. Preliminary findings and other published studies suggest that SRC is a potential link between PKA and phospholipase C. These results suggest a model of the signaling by the membrane glucocorticoid receptor in hypothalamic neuroendocrine cells that includes PKA activation, which in turn activates SRC and phospholipase C, which leads to intracellular calcium release and PKC activation, which causes ERK activation. ERK then stimulates 2-AG synthesis, which is released retrogradely at excitatory synapses to suppress glutamate release.

Disclosures: **G.L. Weiss:** None. **C. Harris:** None. **S. Di:** None. **J.G. Tasker:** None.

Poster

725. Glucocorticoid Actions

Location: Halls B-H

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Program#/Poster#: 725.05/WW15

Topic: F.03. Neuroendocrine Processes

Support: NIH R01 NS039951

DK105826

Title: Corticotropin-releasing hormone (CRH) regulation by acute glucocorticoid receptor activation and restraint stress

Authors: *A. TURNIDGE[1], R. J. HANDA[1,2];
[1]Dept. of Biomed. Sci., Colorado State Univ., Fort Collins, CO; [2]Dept. of Basic Med. Sci., Univ. of Arizona Col. of Med., Phoenix, AZ

Abstract: Activation of the hypothalamic pituitary adrenal (HPA) axis enables homeostatic responses to environmental changes by increasing glucocorticoid (GC) production. Yet, chronic HPA activation alters behaviors in rodents and increases risk for neuropsychiatric disorders in humans, emphasizing the importance of understanding its regulation. The principle regulator of the HPA axis is CRH, a glucocorticoid regulated neuropeptide synthesized and secreted by neurons of the hypothalamic paraventricular nucleus (PVN). Largely through binding to the glucocorticoid receptor (GR), GCs directly down-regulate PVN CRH expression to maintain basal and stress-induced HPA activity within homeostatic limits. Despite its significance, the molecular mechanisms underlying GC negative regulation of CRH remain poorly understood. To inhibit PVN *Crh* expression, GCs can influence *Crh* transcription either through direct DNA binding mechanisms or indirect interactions with other signal transduction pathways. Initially, using immunohistochemistry on CRH-cre:tdTomato (Ai14) mice in which CRH neurons are permanently tagged with a tdTomato fluorphore, we demonstrated expression of GR in virtually all CRH neurons of the PVN. To then determine if GRs are involved in the effects of acute glucocorticoid treatment on HPA activity and *Crh* expression we utilized digital droplet PCR (ddPCR) to allow us to measure, with a high degree of sensitivity and accuracy, absolute levels of CRH mRNA and primary transcript (heterologous nuclear RNA; hnRNA) in micropunched PVN. 48 hrs after adrenalectomy (ADX), male C57bl5/J mice were injected with RU28362 (0.4 mg/kg BW; a selective GR agonist) or Vehicle and then challenged with a 20-minute restraint stress. Mice were sacrificed immediately at the end of restraint (stressed mice) or after removal from their homecage (unstressed mice). Brains were frozen, thick coronal sections were taken, and the paraventricular nuclei were micropunched. Absolute levels of CRH mRNA and CRH hnRNA were measured using ddPCR. Results show that RU282362 treatment decreases CRH hnRNA compared to vehicle treated controls in unstressed animals ($P<0.05$). Following the 20'

restraint stress we also observed a significant decrease in CRH mRNA ($P<0.01$) and hnRNA ($P<0.01$), which was potentiated by RU28362. These data indicate that GCs can act through GR to rapidly suppress HPA activity and, moreover, restraint stress can also rapidly reduce CRH transcription. Our results ultimately suggest that GR plays a role in down-regulating PVN CRH in a context dependent manner.

Disclosures: A. Turnidge: None. **R.J. Handa:** None.

Poster

725. Glucocorticoid Actions

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 725.06/WW16

Topic: F.04. Stress and the Brain

Title: Forebrain glutamatergic, but not GABAergic neurons mediate anxiogenic effects of the glucocorticoid receptor

Authors: *J. HARTMANN[1,2], N. DEDIC[2], M. L. PÖHLMANN[2], A. HÄUSL[2], H. KARST[3], C. ENGELHARDT[2], S. WESTERHOLZ[2], K. V. WAGNER[2], C. LABERMAIER[2], L. HOEIJMAKERS[2], M. KERTOKARIJO[2], A. CHEN[2], M. JOËLS[3], J. M. DEUSSING[2], M. V. SCHMIDT[2];
[1]McLean Hospital, Harvard Med. Sch., Belmont, MA; [2]Max Planck Inst. of Psychiatry, Munich, Germany; [3]Brain Ctr. Rudolf Magnus, UMC Utrecht, Utrecht, Netherlands

Abstract: Anxiety disorders constitute a major disease and social burden worldwide, however many questions concerning the underlying molecular mechanisms still remain open. Besides the involvement of the major excitatory (glutamate) and inhibitory (GABA) neurotransmitter circuits in anxiety disorders, the stress system has been directly implicated in the pathophysiology of these complex mental illnesses. The glucocorticoid receptor (GR) is the major receptor for the stress hormone cortisol (corticosterone in rodents) and is widely expressed in excitatory and inhibitory neurons, as well as in glial cells. However, currently it is unknown which of these cell populations mediate GR-actions that eventually regulate fear and anxiety-related behaviors. In order to address this question, we generated mice lacking the receptor specifically in forebrain glutamatergic or GABAergic neurons by breeding $GR^{flox/flox}$ mice to *Nex-Cre*-mice or *Dlx5/6-Cre* mice, respectively. GR deletion specifically in glutamatergic, but not in GABAergic neurons induced hypothalamic-pituitary-adrenal axis hyperactivity, and reduced fear and anxiety-related behavior. This was paralleled by reduced GR-dependent electrophysiological responses in the basolateral amygdala. Importantly, viral-mediated GR deletion additionally showed that fear expression, but not anxiety, is regulated by GRs in glutamatergic neurons of the BLA. This

suggests that pathological anxiety likely results from altered GR signaling in glutamatergic circuits of several forebrain regions, while modulation of fear-related behavior can largely be ascribed to GR signaling in glutamatergic neurons of the BLA. Collectively, our results reveal a major contribution of GRs in the brain's key excitatory, but not inhibitory neurotransmitter system in the regulation of fear and anxiety behaviors, which is crucial to our understanding of the molecular mechanisms underlying anxiety disorders.

Disclosures: **J. Hartmann:** None. **N. Dedic:** None. **M.L. Pöhlmann:** None. **A. Häusl:** None. **H. Karst:** None. **C. Engelhardt:** None. **S. Westerholz:** None. **K.V. Wagner:** None. **C. Labermaier:** None. **L. Hoeijmakers:** None. **M. Kertokarijo:** None. **A. Chen:** None. **M. Joëls:** None. **J.M. Deussing:** None. **M.V. Schmidt:** None.

Poster

725. Glucocorticoid Actions

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Program#/Poster#: 725.07/WW17

Topic: F.04. Stress and the Brain

Support: Ricerca di Università (Sapienza Università di Roma), grant C26A15PJLW

Title: Hippocampal neurons of dystrophic mdx mice respond differently to acute corticosterone treatment, both *In vitro* and *In vivo*, compared to wild type

Authors: *M. DE STEFANO[1,2], F. COSMI[1], I. BOZZONI[1], P. FRAGAPANE[3];
[1]Dept. of Biol. and Biotech. Charles Darwin, Sapienza Univ. of Roma, Roma, Italy; [2]Inst. Pasteur-Fondazione Cenci Bolognetti, Roma, Italy; [3]Consiglio Nazionale delle Ricerche, Inst. di Biologia e Patologia Molecolari, Roma, Italy

Abstract: Stress induces hypothalamus-pituitary-adrenal axis activation and increase in circulating glucocorticoids (GCs). Major brain target of GC is the hippocampus, in which acute and chronic stressful stimuli induce changes in neuronal activity and synaptic functions, relying on circuit remodelling largely mediated by gene expression modifications. GCs are anti-inflammatory, used as treatment in a variety of pathologies, including the Duchenne Muscular Dystrophy (DMD), a lethal, X-linked disease characterized by progressive muscular wasting consequent to lack of dystrophin (Dp427). Dp427 is a cytoskeletal protein highly represented in muscles, but also expressed by several brain regions, including hippocampus. As a consequence, DMD patients also experience various neurological disorders. In this study, we analysed whether GC treatment affected hippocampal neuron physiology, both *in vitro* and *in vivo*, focusing on the modulation of GC receptor (GR) expression and activation. GR mRNA and protein levels were

analysed in hippocampal neuron cultures from E18 wild type (WT) and dystrophic *mdx* mice, following acute incubation with either 1 μM or 10 μM corticosterone (CORT). In WT mouse neurons, GR mRNA levels significantly increased (1.5 folds) after both treatments, compared to control (vehicle alone). Differently, in *mdx* mouse neurons, mRNA levels increased slightly only after 1μM CORT incubation. Protein levels (Western blot) and intensity of immunolabeling for GR and active *p*GR (phosphorylated) changed accordingly to mRNA results. These first data suggest that the GC-GR intracellular signalling in *mdx* mouse neurons could be less effective compared to WT, negatively affecting GR mRNA synthesis and consequent post-transcriptional modifications. Alternatively, *mdx* mouse neurons could be "pre-sensitized" during gestation by blood-derived GCs of pregnant mothers, which are afflicted by mild myodegeneration and inflammation. This might induce a sort of negative feedback, as described *in vivo*. Acute *in vivo* treatment, gave different results. One hour after CORT injection, levels of *p*GR increased significantly in both WT and *mdx* mouse hippocampi. GR protein levels, instead, significantly decreased in WT mice (negative feedback response), but remained similar to control in *mdx* mice. This result reinforces the idea that GC-GR intracellular signaling, and/or stress modulation by other factors (i.e. sympathetic neuron activity), could be affected in dystrophic mice.

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Poster

725. Glucocorticoid Actions

Location: Halls B-H

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Program#/Poster#: 725.08/WW18

Topic: F.04. Stress and the Brain

Support: CONACYT Grant 256882

Title: Increased serotonin transporter expression by chronic stress in adrenal glands: a glucocorticoid-dependent effect

Authors: ***S. SHANKER**[1], N. SAROJ[2], M. NOYOLA-DIAZ[2], J. A. TERRÓN[2];
[1]FARMACOLOGIA, [2]Pharmacol., CINVESTAV-IPN, Ciudad DE Mexico, Mexico

Abstract: Chronic restraint stress (CRS) has been shown to increase acute stress-induced corticosterone (CORT) secretion in rats through a mechanism involving increased serotonin (5-HT) levels in the adrenal cortex. Similarly, chronic stress increases expression of 5-HT transporter (5-HTT) in the central nervous system. The purpose of the present study was to investigate whether 5-HTT-like immunoreactivity (5-HTT-LI) and expression may also be increased in the adrenal glands as a result of CRS exposure. In addition, we asked the question

whether potential CRS-induced changes in adrenal 5-HTT might be glucocorticoid-dependent. We examined the effect of CRS (20 min/day) as compared to control (CTRL) home cage conditions for 14 days on 5-HTT-LI in adrenal gland sections and 5-HTT protein levels in whole adrenal glands. On the other hand, the effect of a chronic 14-day treatment with CORT (20 mg/kg, s.c. per day for 14 days) as compared to vehicle (VEH; 1 mL/kg, s.c.) was also evaluated on 5-HTT-LI and 5-HTT protein in adrenal glands. 5-HTT-LI and 5-HTT protein levels in adrenal glands were determined, respectively, by immunohistochemistry and Western blot experiments using specific antibodies. CRS decreased body weight gain and relative thymus weight while increasing relative adrenal weight. Furthermore, CRS markedly increased 5-HTT-LI in the adrenal cortex as compared to CTRL treatment; this change involved an increased expression of 5-HTT as an increased amount of protein was detected in adrenals from CRS animals. Interestingly, whereas chronic CORT treatment induced a significant decrease of body weight gain and relative thymus weight, it evoked a significant involution of adrenal glands with respect to VEH treatment. Similar to CRS, chronic CORT treatment induced an increase of 5-HTT-LI in the adrenal cortex as compared to VEH as well as an increased amount of 5-HTT protein in whole adrenal glands. Together, these results suggest that CRS-induced up-regulation of 5-HTT in the adrenal glands is a glucocorticoid-dependent effect that might be associated with the mechanism of endocrine dysregulation leading to increased stress-induced CORT secretion in rats. Whether the adrenocortical 5-HTT could be a target of antidepressant 5-HT reuptake inhibitors remains to be investigated. CRS-induced adrenal gland hypertrophy seems to be a glucocorticoid-independent phenomenon.

Disclosures: S. shanker: None. **N. Saroj:** None. **M. Noyola-Diaz:** None. **J.A. Terrón:** None.

Poster

725. Glucocorticoid Actions

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Topic: G.08. Drugs of Abuse and Addiction

Support: Polish National Science Centre Grant 2013/08/A/NZ3/00848

Title: Opioid alterations of the reward circuit transcriptome involve glucocorticoid pathways within astrocytes

Authors: *R. A. PRZEWLOCKI, S. GOLDA, M. PIECHOTA, U. SKUPIO, M. KOROSTYNSKI, J. BARUT, M. TERTIL;
Mol. Neuropharm., Inst. Pharmacol PAS, Krakow, Poland

Abstract: Our previous study suggested that various drugs of abuse causing alterations of brain gene expression may involve glucocorticoid mechanisms. The aim of the study was to identify using next-generation sequencing the specific transcriptional alterations in the nucleus accumbens (Nac) in opioid-dependent mice and protracted withdrawal mice. We used C57Bl/6J mice injected acutely with morphine, or injected chronically with increasing dose of the drug twice a day. The transcriptome analyses were accompanied by the estimation of corticosterone level in the blood. Acute morphine (20 mg/kg 4h after injection) activated expression of several genes (Fkbp5, Zbtb16, Tsc22d3, Slc2a1, Plin4; FDR<0.1) regulated by GR-receptor, as well as activity-dependent genes (Fos,Fosl2) and group of genes such as Camk1g, Trim2 or Gpr83, which are suggested to be involved in neuronal plasticity. Prolonged treatment with morphine led to significant increase in GR-regulated genes and circadian clock genes (Per1, Per2, Per3) in the Nac of opioid-dependent mice. These changes were positively correlated with the peak of corticosterone in the blood. All the alterations in the transcriptome were transient and absent in protracted withdrawal mice that displayed a depressive–like behavior. In order to identify transcriptional changes in the specific striatal cell types of mice treated with a single dose of morphine or dexamethasone (a GR ligand) we isolated primary neurons and astrocytes utilizing MACS sorting technology. The study revealed that the vast majority of the GR genes are altered in astrocytes but not in neurons. The study indicates that stress and circadian systems in the Nac may affect the reward circuit using GR-dependent molecular pathways within astrocytes. The mechanism may be essential for development of opioid tolerance, dependence and addiction, and predict molecular targets for future therapies.

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Poster

725. Glucocorticoid Actions

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Topic: F.03. Neuroendocrine Processes

Support: NIH Grant GM63904

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Title: Characterizing HPI axis response to acute stress using mutant zebrafish strains in the HPI axis receptors

Authors: *H. LEE[1], T. L. SCHWAB[2], R. G. KRUG, II[3], M. R. SERRES[2], A. N. SIGAFOOS[2], B. N. SUNDBERG[2], C. E. BULLARD[2], B. C. BERRY[2], K. J. CLARK[3,2];
[1]Mayo Grad. Sch. - Neurobio. of Dis., Rochester, MN; [2]Biochem. and Mol. Biol., Mayo Clin., Rochester, MN; [3]Neurobio. of Dis., Mayo Grad. Sch., Rochester, MN

Abstract: A hallmark pathophysiological change in neuropsychiatric disorders is alterations in the hypothalamic-pituitary-adrenal (HPA) axis activity. The HPA axis mediates vertebrate-specific systemic stress response (SR) through biphasic glucocorticoid receptor (GR) signaling comprising rapid non-genomic and slower genomic responses. It is largely unknown how changes in non-genomic GR response lead to lasting alterations in genomic response. Leveraging that the HPI (interrenal cells) axis in zebrafish is a functional homologue to the HPA axis, we hypothesized that zebrafish locomotor response to hyperosmotic stimulation (100 mM NaCl) is dependent on the HPI axis and rapid non-genomic GR signaling. This larval zebrafish locomotor assay provides a versatile platform that can dissect molecular components of non-genomic glucocorticoid signaling independently from any genomic response. By measuring locomotor response, genomic GR activation, cortisol levels, and drug response to selected antagonists using zebrafish mutants in the HPI axis, we have demonstrated that the locomotor response is dependent on *mc2r* (adrenocorticotropic hormone receptor). Both GR somatic mutants injected with TALENs and GR germline mutants have significantly decreased genomic response shown by the level of GR transcripts and glucocorticoid response element activation. However, rapid locomotor response to hyperosmotic stress is not decreased in GR germline mutants whereas significantly decreased in GR-targeting TALEN-injected fish. We are investigating translational GR isoforms potentially responsible for non-genomic response in germline mutants by targeting alternative loci in GR with different TALENs and TALEN-mediated precision knock-in strategies. Dissecting non-genomic and genomic GR response with genetic perturbation may lead to an establishment of a versatile assay system to investigate vertebrate SR and identification of components in non-genomic GR response.

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Poster

725. Glucocorticoid Actions

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Topic: F.04. Stress and the Brain

Support: CONACYT Grant 256882

Title: Effect of chronic corticosterone treatment on the amount and distribution of serotonin 5-HT7 receptors in rat adrenal glands

Authors: *N. SAROJ, S. A. MENDOZA-CONTRERAS, S. SHANKER, M. NOYOLA-DIAZ, J. A. TERRÓN;
Pharmacol., CINVESTAV-IPN, Ciudad de Mexico, Mexico

Abstract: Chronic restraint stress (CRS) increases acute stress-induced corticosterone (CORT) secretion in rats apparently through an ACTH-independent mechanism involving 5-HT7 receptors, and also increases the amount of 5-HT7 receptors in the adrenal cortex. Furthermore, ectopic expression of adrenocortical 5-HT7 receptors has been reported in human ACTH-independent glucocorticoid-producing adrenal tumors. In this study we asked the question whether CRS-induced changes in adrenocortical 5-HT7 receptors are glucocorticoid-dependent. The effect of a chronic 14-day treatment with CORT (50 mg/kg, s.c., per day) as compared to that of vehicle (VEH; 1 mL/kg, s.c., per day) was evaluated on: 1) 5-HT7 receptor-like immunoreactivity (5-HT7-RLI) in adrenal gland sections; 2) 5-HT7 receptor protein in whole adrenal glands; and 3) baseline (0 min) and restraint (10 and 30 min)-induced ACTH and CORT secretion. One day after completion of treatments (on day 15), one group of animals of both treatments were anesthetized and perfused with fixative via the ascending aorta; another group of VEH- and CORT-treated animals were decapitated and trunk blood, adrenal glands and thymus were collected. 5-HT7-RLI and 5-HT7 receptor protein levels in adrenal glands were determined by immunohistochemistry and Western blot assays, respectively, using specific antibodies; hormone levels were measured using commercially available Elisa kits. Chronic CORT treatment significantly decreased body weight gain and thymus weight; in contrast to observations in chronically stressed animals however, chronic CORT evoked a significant involution of adrenal glands with respect to VEH treatment. Chronic CORT treatment induced a modest but noticeable increase of 5-HT7-RLI in the adrenal cortex as compared to VEH with the signal predominantly located in adrenocortical *zona fasciculata* cells. In addition, a significant increase in the amount of adrenal 5-HT7 receptor protein was detected in CORT- as compared to VEH-treated animals. Finally, chronic CORT treatment evoked a significant decrease of stress-induced secretion of ACTH at 10 min of restraint along with a trend towards a decrease of ACTH levels at 0 min (baseline) and 30 min of restraint; also, significantly higher CORT levels were detected at 0 min, but not at 10 and 30 min of restraint in CORT-treated animals. Results support the notion that CRS-induced increase of adrenocortical 5-HT7 receptors is partly dependent on glucocorticoids; the development of hypercorticolemia during chronic stress however may involve additional mechanisms further to increased 5-HT7 receptor expression in adrenocortical steroidogenic cells.

Disclosures: **N. Saroj:** None. **S.A. Mendoza-Contreras:** None. **S. shanker:** None. **M. Noyola-Diaz:** None. **J.A. Terrón:** None.

Poster

726. Early-Life Stress: Perinatal

Location: Halls B-H

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Topic: F.04. Stress and the Brain

Support: R01-MH091451

R37-HD083217

R01-AA023181

Title: Early life trauma has life-long consequences for sleep

Authors: *M. P. LEWIN[1,3,4], R. M. SULLIVAN[3,2], D. A. WILSON[3,2];
[1]NYU, New York City, NY; [2]NYU, New York, NY; [3]Emotional Brain Inst., Nathan Kline Inst., Orangeburg, NY; [4]Sackler, NYU Sch. of Med., New York, NY

Abstract: Early life adversity is linked increased the incidence of later life psychiatric diseases related to anxiety, depression, anger, and antisocial behaviors. Individuals who have experienced trauma in early life are also reliably found to have a higher incidence of sleep problems, however, these human data generally rely on self-report and actigraphy, thus they provide limited insight into potential specific sleep mechanisms affected, and it remains unclear whether sleep disturbance is a consequence of psychiatric outcomes, or rather, a cause. Here, we used a naturalistic model of early life trauma in the rat where mothers are supplied insufficient wood shavings for nest building during specific postnatal periods of pup development (from 8-12 days old). The dearth of resources produces aberrant maternal behavior and harsh, mistreatment of the pups. Though they do not suffer from abnormal postnatal weight gain, pups in this low shavings (LS) condition exhibit robust behavioral and neurological disturbances across the lifespan, including depressive-like behavior, disordered social behavior, impaired emotional learning (fear conditioning), as well as altered neural function. We monitored sleep-wake function and circadian rhythms at 3 different points across the lifespan, recording from 7 age-matched LS-CON pairs in each age group: adolescence (PN 21-30), young adulthood (2-4 months old), and older adulthood (6-10 months old), using telemetry cortical local field potential recordings and EMG. Data were collected continuously for each pair for up to 72 hrs. Our initial results demonstrate not only a difference in sleep physiology after early life trauma, but a trajectory of sleep dysfunction that changes across the lifespan. Postnatally abused animals show abnormally consolidated NREM sleep in early life (longer bouts of NREM sleep, greater NREM delta power) which shifts to NREM deficiency (NREM fragmentation, reduced NREM delta) in older adulthood. Future experiments will examine whether this sleep pathology may be bidirectionally

related to the neurobehavioral deficits associated with early life trauma, and specifically whether sleep repair can ameliorate its lasting cognitive/emotional consequences.

Disclosures: **M.P. Lewin:** None. **R.M. Sullivan:** None. **D.A. Wilson:** None.

Poster

726. Early-Life Stress: Perinatal

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Program#/Poster#: 726.02/XX1

Topic: F.04. Stress and the Brain

Support: HD083217

MH091451

Title: Effects of maternal maltreatment on parvalbumin cells in the basolateral amygdala

Authors: ***A. N. SANTIAGO**[1,2], K. LIM[1], M. OPENDAK[1,2], C. AOKI[1], R. SULLIVAN[1,2]; [1]New York Univ., New York, NY; [2]Nathan Kline Inst., Orangeburg, NY

Abstract: Early life trauma, particularly during the developmental sensitive period for infant attachment, is a major risk factor for later-life vulnerability to psychiatric disorders characterized by heightened fearfulness. The basolateral amygdala (BLA), long known for its integral role in threat response, has recently been shown to be hyper-responsive following early life abuse from the caregiver. However, the anatomical substrates that mediate increased responsiveness of the BLA at a synaptic level are unknown. Here, we use a rodent model of maternally-induced trauma to investigate the hypothesis that abuse during critical periods of neurodevelopment directly alters the inhibitory neurocircuitry for disinhibition of the BLA. Fast-spiking parvalbumin positive (PV+) inhibitory interneurons are known to play a major role in defining critical periods of neurodevelopment and have recently been shown to play a critical role in BLA fear response. A class of extracellular molecules referred to as perineuronal nets (PNNs), have been shown to end critical periods of neurodevelopment by locking axo-somatic synapses onto PV+ somata, thus preventing synaptic rearrangement onto these cells. Dissolution of PNNs has been shown to reopen critical windows for plasticity, allowing synaptic rearrangement onto PV+ cells to restore healthy function following deprivation. We hypothesize that early life abuse induces increased inhibitory synaptic input onto PV+ cells in the BLA, thus facilitating disinhibiton, which becomes locked by PNN development. Here, we report the effects of 5 days of maternally-induced trauma on amygdala hyperactivity in the BLA of rats at 3 specific ages: post-trauma (PN14), during post-trauma recovery (PN18) and at weaning (PN23). We then used light

microscopy to measure PNN formation over PV+ cells, as well as electron microscopy to quantify axo-somatic input onto PV+ neurons. Group differences are reported.

Disclosures: **A.N. Santiago:** None. **K. Lim:** None. **M. Opendak:** None. **C. Aoki:** None. **R. Sullivan:** None.

Poster

726. Early-Life Stress: Perinatal

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Topic: F.04. Stress and the Brain

Support: MATRICS EU FP7 603016

TACTICS EU FP7 278948

Title: Chronic early life stress reduces cognitive flexibility and orbitofrontal cortex axonal integrity in mid-aged mice.

Authors: ***C. A. OOMEN**[1,2], H. AMIRI[2], H. J. KRUGERS[1], J. C. GLENNON[2];
[1]SILS - Ctr. for Neurosci., Univ. of Amsterdam, Amsterdam, Netherlands; [2]Cognitive Neurosci., Donders Institute, Radboud Univ. Med. Ctr., Nijmegen, Netherlands

Abstract: Adversity early in life has been found to increase the risk for developing stress-related disorders in adulthood, such as depression and anxiety disorders. One of the major moderators in this is the quality of parental care. In rodents, disrupting maternal care can result in a stress-sensitive phenotype and learning and memory deficits, often studied at the level of the hippocampus. However, less is known about how early life stress impacts the prefrontal cortex and executive functions such as cognitive flexibility, especially in older animals.
In order to address this, we studied the effects of chronic early life stress (ELS) on reversal learning and orbitofrontal cortex structure in mice. ELS was induced by limiting the availability of nesting and bedding material from postnatal day (PND) 2-9 which results in fragmented maternal care and a stress sensitive phenotype in the offspring later in life. Between 11 and 15 months of age, ELS and control mice were tested on instrumental conditioning, reward-based visual discrimination learning and subsequent reversal learning using a touchscreen-equipped operant chamber. After testing behaviour, mice were scanned using an 11.2T MRI scanner. Single voxel ^{1}H magnetic resonance spectroscopy (MRS) and diffusion tensor imaging (DTI) experiments were performed to assess potential metabolic and structural changes in the orbitofrontal cortex and dorsomedial striatum. Finally, detailed morphological analysis of these

areas was performed post-mortem, by 3D reconstruction and analysis of dendritic complexity of individual (Golgi-stained) neurons.
We found that ELS does not impair acquisition of a visual discrimination paradigm. However, ELS reduces cognitive flexibility as reflected by increased correction errors during reversal learning of the acquired contingencies. This was paralleled by a decrease in the fractional anisotropy (FA) in the orbitofrontal cortex, indicative of changes in axonal architecture, while no differences in dendritic morphology were found. We conclude that ELS has long-term effects on executive function, which are present at mid-age, possibly mediated by differences in axonal projections.

Disclosures: **C.A. Oomen:** None. **H. Amiri:** None. **H.J. Krugers:** None. **J.C. Glennon:** None.

Poster

726. Early-Life Stress: Perinatal

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Program#/Poster#: 726.04/XX3

Topic: F.04. Stress and the Brain

Support: University at Albany start-up funds

Title: Effects of early postnatal undernutrition on affective behavior and conditioned fear in male and female Sprague Dawley rats

Authors: *R. M. DE GUZMAN, L. M. COLON, A. M. POULOS, J. L. WORKMAN; Psychology, Univ. at Albany, State Univ. of New York, Albany, NY

Abstract: Undernourishment during critical periods of brain development has long-term consequences for brain and behavior. In humans, early childhood malnutrition increases depression and suicidal thoughts in adolescents, as well as externalizing behaviors compared with well-nourished children. In rats, early-life undernutrition restricts growth and impairs spatial navigation. The hippocampus, which is involved in memory, stress, and affect, is particularly affected. For instance, early-life undernutrition decreases the total number of granule cells and cell proliferation in the dentate gyrus. However, rodent studies investigating hippocampal plasticity following early-life undernutrition have mainly investigated males. This study aimed to determine the behavioral and neural changes that occur following early postnatal undernutrition in male and female Sprague Dawley rats during adolescence and adulthood. Many rodent models of early-life undernutrition restrict food or nutrient availability to dams, which can alter maternal care. In this study, dams underwent either thelectomy (surgical removal of teats to inhibit milk letdown), sham surgery, or no surgery (control). During the postnatal period,

offspring born to thelectomized and sham rats were rotated every 12 h to restrict feeding and offspring from control dams were not rotated and had unrestricted feeding. All dams had ad libitum access to food. Maternal care did not significantly differ between groups. We tested whether early postnatal undernutrition affected fear acquisition and extinction in fear conditioning and affective behaviors in the forced swim test and open field. Early postnatal undernutrition reduced body mass throughout lifespan and reduced distance traveled in the open field. When distance traveled was controlled for, early postnatal undernutrition increased entries into and time spent in the center of the open field in adolescents only, which suggests early postnatal undernutrition reduces anxiety-like behavior. Data on fear conditioning, forced swim test, and hippocampal neurogenesis will be presented. This research will reveal how early-life undernutrition influences behavior and brain plasticity in males and females and has implications for humans who experienced early life undernourishment.

Disclosures: R.M. De Guzman: None. **L.M. Colon:** None. **A.M. Poulos:** None. **J.L. Workman:** None.

Poster

726. Early-Life Stress: Perinatal

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Program#/Poster#: 726.05/XX4

Topic: F.04. Stress and the Brain

Support: Fac Med UNAL 2016

Title: Relation between maternal separation during breastfeeding in rats and giardia infection

Authors: M. F. GERENA-CRUZ[1], D. G. GARCÍA LAGUNA[3], *Z. DUENAS[2]; [1]Ciencias Fisiológicas, [2]Univ. Nacional De Colombia, Bogota DC, Colombia; [3]Ciencias Fisiológicas, Univ. Nacional de Colombia, Bogotá D.C., Colombia

Abstract: It has been reported in studies with rodents that maternal separation during breastfeeding (MSDB) generates changes at behavioral and physiological levels of the offspring. The breastfeeding, it has been shown that it provides benefits as a protective factor on gastrointestinal parasitic infections. Currently there are no reports correlating the disruption of breastfeeding caused by maternal separation and the development of intestinal infection for Giardia. The aim of this study was to establish the effect of maternal separation during breastfeeding on the Giardia infection in Wistar rats. MSDB protocol was performed from postnatal day 1 to postnatal day 21 for 360 minutes a day (180 in the morning and 180 in afternoon) with reverse light cycle. On postnatal day 22, the pups were distributed by sex and

treatment; Group 1 (MSDB with infection), Group 2 (MSDB without infection), Group 3: (No MSDB with infection) and Group 4 (No MSDB without infection). Infection induction at postnatal day 25 was performed through oral ingestion of Giardia Lambia cysts isolated. The course of infection was analyzed by direct quantification of cysts in the feces, which were collected during the days 5, 10, 15 and 20 post-infection. Intestine samples were taken for future analysis. The results show that rats with MSDB have more infection compared with non MSDB rats ($p < 0.05$). The analysis for sex of the release of cysts indicates that MSDB males have more infections than control males ($p<0.05$). Additionally, was found statistically significant differences for sex, among the four study groups with their respective controls, between group 1 Vs. group 2 and group 3 Vs. group 4 ($P <0.05$). The analysis of intestinal tissue of rats is currently under study in order to analyze possible histological changes in the small intestinal mucosa based on the infection. Preliminary results suggest that maternal separation with the interruption of breastfeeding predisposes to develop a Giardia infection, increasing the risk of disease in the intestinal tissue. These data supports that breastfeeding as a protective factor in the development of intestinal infections by this parasite.

Disclosures: **M.F. Gerena-Cruz:** None. **D.G. García Laguna:** None. **Z. Duenas:** None.

Poster

726. Early-Life Stress: Perinatal

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 726.06/XX5

Topic: F.04. Stress and the Brain

Title: Early-life obesity increases susceptibility to post traumatic stress and disrupts hippocampal structure

Authors: *J. D. VEGA-TORRES[1], P. KALYAN-MASIH[2], C. MILES[2], E. HADDAD[2], S. RAINSBURY[2], M. BAGHCHECHI[2], A. OBENAUS[2], J. D. FIGUEROA[2];
[1]Basic Sci., [2]Loma Linda Univ. Sch. of Med., Loma Linda, CA

Abstract: Early-life traumatic stress and obesity co-occur frequently and have been identified as major risk factors for anxiety disorders. Surprisingly, preclinical studies examining how obesity disrupts the ability of the brain to cope with traumatic stress are lacking. The objective of this study was to determine whether an obesogenic Western-like high-fat diet (WD) predisposes rats to posttraumatic stress. Adolescent Lewis rats (postnatal day, PND, 28) were fed *ad libitum* during eight weeks with either the experimental WD diet (41.4% kcal from fat) or the control diet (CD; 16.5 % kcal from fat). Posttraumatic stress responses were evaluated at one week following exposure to a predator odor threat by using standard behavioral paradigms. The

elevated plus maze and the open field test revealed increased anxiety-like behaviors in the rats consuming the WD when compared to control animals at one-week posttraumatic stress ($p < 0.05$). Magnetic resonance imaging (MRI) showed a significant 20% reduction in the total hippocampal volume of animals fed the WD when compared to controls. The reduced hippocampal formation was associated with increased behavioral indices of anxiety, neuroinflammation, and FKBP51 protein levels. Notably, we found asymmetric hippocampal vulnerabilities to the WD and stress, particularly in the ventral and left hippocampus. This study shows how WD intake during early life impacts key substrates implicated in posttraumatic stress disorder (PTSD). Understanding how trauma and obesity affect the developmental trajectories of the stress neurocircuitry is critical, as stress susceptibility imposes a marked vulnerability to neuropsychiatric disorders.

Disclosures: **J.D. Vega-Torres:** None. **P. Kalyan-Masih:** None. **C. Miles:** None. **E. Haddad:** None. **S. Rainsbury:** None. **M. Baghchechi:** None. **A. Obenaus:** None. **J.D. Figueroa:** None.

Poster

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Program#/Poster#: 726.07/XX6

Topic: F.04. Stress and the Brain

Title: Consequences of neonatal maternal separation on the efficacy of the oxytocinergic control of pain

Authors: ***M. MELCHIOR**, P.-E. JUIF, N. PETIT-DEMOULIÈRE, V. CHAVANT, Y. GOUMON, V. LELIÈVRE, A. CHARLET, P. POISBEAU;
INCI, Strasbourg, France

Abstract: Prematurity is a huge public health issue, since it concerns as many as 1/10 birth in the world. Most of the time, an integration in intensive care units is required for these preterm newborns, where they are submitted to repeated painful procedures and to maternal separation (MS) possibly altering the mother-infant link. In both human and animal studies, these perinatal events are known to have detrimental effects on the development of the newborn, especially on brain maturation. Clinical data strongly suggests that preterm babies are indeed at high risk of developing, at adulthood, neuropathologies affecting cognitive functions, chronic anxiety, depressive states, chronic pain and visceral dysfunctions. Hypothalamic changes, among other mechanisms, may explain the high prevalence of anxiety, stress disorders, visceral hypersensitivity, cognitive and social impairment seen after MS. We hence chose to study the consequences of MS on hypothalamic functions, with a particular interest in oxytocin (OT), a

neurohormone modulating pain responses as well as regulating social and maternal behavior. MS is performed by separating Wistar rat pups from their mother 3 hours per day, between postnatal day 2 and 12 (P2-P12), mimicking some developmental and environmental components of preterm birth. Combining behavioral, electrophysiological and molecular studies, we identified a strong hypersensitivity to mechanical and thermal hot stimulation and a clear dysfunction of hypothalamic descending controls of pain after MS. In vivo electrophysiological recordings of wide dynamic range neurons in the spinal cord of MS rats revealed that OT failed to exert an efficient inhibitory control on nociceptive processing. This result was further confirmed on freely-moving animals since stress-induced analgesia mediated by OT receptors could not be observed. In carrageenan-induced inflammatory pain, OT inhibitory controls were also inefficient since MS animals displayed more intense pain symptoms and had a longer-lasting expression of them.
Altogether, behavioral and electrophysiological results strongly suggest that the descending inhibitory control of pain is impaired in adult rats previously submitted to MS. Lack of efficacy of OT in non-painful and painful stress conditions may easily explain several maladaptive behaviors observed after MS in these animals. The spinal mechanisms underlying these alterations are currently under investigation but may also affect supraspinal structures expressing OT receptors.

Disclosures: **M. Melchior:** None. **P. Juif:** None. **N. Petit-Demoulière:** None. **V. Chavant:** None. **Y. Goumon:** None. **V. Lelièvre:** None. **A. Charlet:** None. **P. Poisbeau:** None.

Poster

726. Early-Life Stress: Perinatal

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Program#/Poster#: 726.08/XX7

Topic: F.04. Stress and the Brain

Support: RFBR Grant 16-34-00253

Title: Early-life predictors of the effects of repeated maternal deprivation on the development of 129Sv mice

Authors: *O. V. BURENKOVA, E. A. ALEKSANDROVA, I. Y. ZARAYSKAYA;
P.K. Anokhin Res. Inst. of Normal Physiol., Moskva, Russian Federation

Abstract: Maternal care during early life influences physiology and behavior of adult animals via epigenetic mechanisms. In particular, maternal deprivation of neonatal mice and rats has long-term dramatic impact on offspring development. Previously we revealed immediate and

long-lasting adverse effects of repeated maternal deprivation (45 min daily on postnatal days (PND) 3-6) on physiology and behavior of 129Sv mice. In the present work, we investigated hormonal and behavioral factors which could cause these changes.
We analyzed two early-life stress markers: pup ultrasonic vocalizations (USVs) rates on PND 3 & 6, and the levels of corticosterone in pups on PND 7. In addition, we examined maternal behavior in the home cage for 30 min before deprivation and for 30 min immediately after the reunion with the pups on PND 3 and 6. This allowed us to reveal the correspondence between distinct acoustic properties of different signal types and the types of maternal behavior.
Maternal deprivation for 45 min/day from PND 3 to PND 6 resulted in changes in maternal behavior, which was accompanied by hormonal and behavioral stress response in 129Sv pups. Specifically, we found an increase in corticosterone levels and USV rates during deprivation in comparison with baseline levels and with non-deprived pups. It is important to emphasize that the elevation of corticosterone levels and USV rates showed no recovery to the baseline levels for 30 min after the reunion. In addition, the increase in USV rates from PND 3 to PND 6 was revealed in deprived pups, but not in non-deprived pups.
Our results suggest that hormonal and behavioral responses described above might be the early-life predictors of the long-term adverse effects of repeated 45-min maternal deprivation on the development of 129Sv mice.

Disclosures: O.V. Burenkova: None. **E.A. Aleksandrova:** None. **I.Y. Zarayskaya:** None.

Poster

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Topic: F.04. Stress and the Brain

Support: AFIP

FAPESP grant 2014/22395-0

CAPES

Title: Increased corticostreone response on postnatal day 11 does not alter emotional or hormonal stress responses of adult male rats: role of maternal care

Authors: *D. SUCHECKI, A. CONSOLI, R. CABBIA;
Univ. Federal de Sao Paulo, Sao Paulo, Brazil

Abstract: Disruptions of mother-infant relationships during the stress hyporesponsive period (SHRP) can alter the programming of behavioral and physiological responses to stress in adulthood. We investigated whether sustained corticosterone secretion during the SHRP induced by maternal deprivation on postnatal day (PND) 11 altered behavioral and hormonal stress responses in adult male rats. To this purpose, 33 mixed sex litters were used: 16 were undisturbed (control group = CTL) and 17 were deprived from their mothers for 24 h (deprived group = DEP) on PND 11. Two hours before the end of the deprivation period, one half of the litters were injected with saline (0.1 ml/10 g of body weight) to induce an intense and sustained CORT secretion (DEP+SAL, N = 7), whereas the other half was not stimulated (DEP+NSAL, N = 10). NDEP litters were also distributed into NSAL (N = 8) and SAL subgroups (N = 8). At the end of the deprivation period or immediately after the SAL injection for NDEP group, litters and mothers were reunited and maternal behavior was assessed for 1 h at 10:00 h, 14:00 h and 17:30 h. Every 3 min, the cage was observed and the predominant behavior of the mother was registered, making up for 20 observations/h. Behavioral tests begun on PND 70, when males were assessed in the following tests: novelty suppressed feeding (measures the motivation and anxiety to eat in novel environments), sucrose negative contrast test (measures the capacity of the animal to perceive a sudden change in hedonic value of a palatable solution), social investigation (evaluates the motivation to explore an unknown conspecific) and elevated plus maze (classic test of anxiety-like behavior), after which rats were decapitated in four different time-points for blood sampling: Basal (not-tested in the behavioral tasks) or 15 min, 45 min or 70 min. The main results of this study were: 1) Maternal deprivation (regardless of the stressful stimulus) increased arched-back nursing and anogenital licking/grooming in the first hour upon reunion of the litters and mothers; 2) DEP+SAL males displayed smaller latency to start eating in the NSF test, but the amount consumed was the same for all groups; 3) There were no changes in the other behavioral tests; 4) All groups had a similar stress response profile, with the peak corticosterone secretion occurring 15 min after the stressor. These findings suggest that increased maternal care induced by separation of the litters from their mothers for 24 h may have protected adult animals from displaying overtly behavioral and hormonal stress responses.

Disclosures: **D. Suchecki:** None. **A. Consoli:** None. **R. Cabbia:** None.

Poster

726. Early-Life Stress: Perinatal

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Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 726.10/XX9

Topic: F.04. Stress and the Brain

Title: Alternation of fundamental frequency of ultrasonic vocalizations by neonatal rats in response to distance from their mother

Authors: *M. NAKAMURA[1], M. TANICHI[2], H. TODA[2], S. MITSUYOSHI[1], S. SHINOHARA[1], Y. OMIYA[3], K. SHIMIZU[4], A. YOSHINO[2], S. TOKUNO[1];
[1]Verbal analysis of pathophysiology, The Univ. of Tokyo, Yokohama-shi, Japan; [2]Dept. of Psychiatry, Natl. Defence Med. Col., Tokorozawa-shi, Japan; [3]PST inc., Yokohama-shi, Japan; [4]Div. of Behavioral Sci., Natl. Defence Med. Col. Res. Inst., Tokorozawa-shi, Japan

Abstract: Neonatal rats elicit caregiving behavior of their mothers using ultrasonic vocalizations (USVs). Separation of neonatal rats from their mothers can cause severe impact on their survival therefore it is assumed that separated neonatal rats will change their behavior to call their mothers according to estimated distance from their mother.
We recorded USVs of neonatal rats with varying distance from their mothers and analyzed changes in features of the USVs according to the distance. A neonatal rat and its mother were put in two cages separately. In first half of the recording we gradually increased the distance between the two cages. In latter half of the recording, we gradually decreased the distance between the two cages. We recorded the USVs for five minutes per every condition (distance). We evaluated the USVs with respect to their fundamental frequency.
We found that USVs of higher fundamental frequency were observed more often as we increase the distance between the two cages. In contrast, we confirmed that USVs of higher fundamental frequency were decreased as the two cages got closer in the latter half of the recording. Average of fundamental frequency also altered responding to this tendency whence the average peaked when the distance was largest.
These results infer that the change in the shift of the fundamental frequency of USVs by neonatal rats does not depend much on duration of separation from their mothers if the dependency on duration of separation is monotonic. Also behavior of the mother rats altered depending on the distance from their pups. These findings suggest that interaction between neonatal rats and their mothers separated from each other change their phase of calling and searching with their estimated distance. The results imply a possibility of predicting psychological stress on neonatal rats from their USVs.

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Poster

726. Early-Life Stress: Perinatal

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Topic: F.04. Stress and the Brain

Support: NARSAD Young Investigator's Award

Title: Perinatal SSRI exposure affects social behavior and related neurobiology in juvenile rat offspring

Authors: *M. GEMMEL[1], M. HAZLETT[1], E. CSÁSZÁR[3], C. VESEL[1], S. DE LACALLE[2], J. PAWLUSKI[1,4];
[1]Dept. of Biol. Sci., [2]Dept. of Biomed. Sci., Ohio Univ., Athens, OH; [3]Dept. of Reproductive Toxicology, Inst. of Exptl. Pharmacol. and Toxicology, Slovak Acad. of Sci., Bratislava, Slovakia; [4]Univ. of Rennes 1, Rennes, France

Abstract: Selective serotonin reuptake inhibitor medications (SSRIs) are the most common antidepressant medications for treatment of maternal mood disorders during the perinatal period. SSRIs cross the placental barrier and are present in breast milk, suggesting an impact on offspring development. Previous clinical research shows effects of SSRI exposure on children's social behavior; however, further work is needed to determine how perinatal SSRI exposure may affect social behaviors and behavior-related neurobiology. The aim of the present study was to determine how perinatal exposure to fluoxetine, a popular SSRI used during the perinatal period, affects social play behaviors and related neurobiology in the hippocampus and prefrontal cortex of juvenile male and female rat offspring. To mimic aspects of maternal depression, prior to breeding, Sprague-Dawley rat dams were subjected to chronic unpredictable stress, and were treated with fluoxetine (10mg/kg/day) or vehicle via oral administration from gestational day 10 to postnatal day 21. Juvenile male and female offspring from the following four groups were used: 1. Control+Vehicle, 2. Control+Fluoxetine, 3. Preconception Maternal Stress+Vehicle, 4. Preconception Maternal Stress+Fluoxetine. Offspring underwent a social interaction test one week after weaning, and brains were preserved for immunohistochemical work. Results show that perinatal fluoxetine exposure, regardless of maternal stress, had a more remarkable effect on juvenile female offspring resulting in increased social behaviors, increased presynaptic immunoreactivity, via synaptophysin, in the dentate gyrus of the hippocampus and decreased postsynaptic immunoreactivity, via PSD95, in the prefrontal cortex. Perinatal fluoxetine exposure also increased social behavior in juvenile male offspring, but to a lesser degree. Exposure to maternal stress prior to conception had a more pronounced effect on juvenile male offspring resulting in decreased presynaptic immunoreactivity in the dentate gyrus of the hippocampus and decreased postsynaptic immunoreactivity in the prefrontal cortex. This work

indicates an effect of perinatal SSRI exposure on brain and behavior, but that these effects are often sexually differentiated. Further work will investigate other measures of plasticity as well as how these early life exposures affect the hypothalamic-pituitary-adrenal system. Investigating the impact of SSRI exposure on neurobehavioral development will further our understanding of the benefits and risks of these medications during the perinatal period.

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Poster

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Topic: F.04. Stress and the Brain

Support: NIMGS COBRE NIH

Title: Sex specific cognitive deficits following early life stress: A role for parvalbumin in the orbitofrontal cortex

Authors: ***H. GOODWILL**, S. LIN, G. MANZANO-NIEVES, A. COHEN, K. BATH; Brown Univ., Providence, RI

Abstract: Adverse early life experiences influence emotional development and increase the risk for and severity of affective pathology. Women are at increased risk of developing stress-associated pathology, and have a two-fold higher risk of developing PTSD, anxiety disorder, and depression when compared with men. In a mouse model of early life stress (ELS), we found that female mice, but not male mice, go on to develop a depressive-like phenotype. Depressive pathology is highly comorbid with cognitive impairments and inflexibility, which is thought to result predominantly from altered frontal lobe function. The prefrontal cortex (PFC), which orchestrates the integration of cognitive and emotion through cortical and subcortical pathways, is especially sensitive to ELS. However, it is largely unknown whether ELS effects cognitive function in a sexually dimorphic manner, and what the underlying mechanisms of disruption are. Here, we use a combination of behavioral, molecular, and optogenetic approaches to examine a possible mechanism underlying sex-specific vulnerability following ELS in mice. Using an attentional set-shifting task, we found that female mice exposed to ELS were impaired on the rule-reversal phase of learning ($p < 0.01$), and observed no differences between groups during initial rule learning or extra-dimensional rule shifts. Fast spiking (FS) GABAergic interneurons containing the calcium binding protein Parvalbumin (PV), have been implicated in PFC-

dependent cognitive functioning, and have been shown to be sensitive to ELS. However, their specific role in reversal learning and cognitive function following ELS remain unclear. Here, we find a decrease in PV and GAD67 mRNA levels in the orbitofrontal cortex (OFC) of female mice following ELS. To test a potential role for PV interneurons in reversal learning, we employed an optogenetic approach using halorhodopsin to selectively silence PV+ cells in the OFC during different test phases of the attentional set shifting task. Findings related to these experiments will be discussed, and have implications for understanding the molecular and cellular mechanisms underlying stress-induced impairments in cognitive function, and sex differences in risk for pathology development.

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Poster

726. Early-Life Stress: Perinatal

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Topic: F.04. Stress and the Brain

Support: Robert and Nancy Carney Fund for Scientific Innovation

Norman Prince Neuroscience Institute Translational Research

National Science Foundation Graduate Research Felloships Program

Title: Early life stress alters the development of the fear recall and expression in pre-adolescent mice

Authors: *G. MANZANO-NIEVES[1], K. G. BATH[2];
[1]Dept. of Neuroscience, Brown Univ., Providence, RI; [2]Dept. Cognitive, Linguistic and Psychological Sci., Brown Univ., Providence, RI

Abstract: Acute traumatic events and/or prolonged stress incurred early in life increase the risk of developing anxiety and depressive-like behaviors in both humans and animal models. However, the effect of early life stress (ELS) on neural development, and their consequences on circuit activation are not well understood. To investigate the effect of ELS on the development of the conditioned fear circuit we took advantage of an ELS paradigm that consisted of reducing maternal access to bedding and nesting materials during postnatal days 4 to 11 (Rice CJ et al., 2008). Subsequently, we investigated the development of fear learning and expression in control reared and ELS mice using an auditory fear conditioning paradigm. Briefly, male and female

mice were exposed to six tones (30s), each of which co-terminated with a foot-shock (1s; 0.57mA) on postnatal day (P) 16, 21, 28, or 50. Interestingly, at postnatal day 21 both ELS reared mice showed significantly diminished levels of freezing during recall testing at 6 hrs. (% time freezing: 45% Controls vs. 17% ELS; $P < 0.01$) and 24 hrs. (% time freezing: 65% Controls vs. 22% ELS; $P < 0.01$) but not at 7 days (% time freezing: 62% Controls vs. 53% ELS; $P = 0.33$) post conditioning. To study the changes in the development of the fear circuit induced by ELS we used a combination of distinct immunohistochemical markers to study late developing neurons. Interestingly, we found that P21 ELS animals had a significantly increased number of parvalbumin positive neurons (PV+; late differentiating inhibitory neurons) when compared to controls at P21 and ELS animals at P16 in both the amygdala and hippocampus but not in the medial prefrontal cortex (mPFC). This data suggests that ELS animals may be undergoing early maturation of subcortical, but not cortical structures, which may be causing an impairment in the mPFC - amygdala communication, resulting in the inability of ELS mice to express the conditioned memory at normal levels. To test if ELS animals could express a freezing response when basal lateral amygdala (BLA) activity is increased during each tone of fear conditioning, we optogenetically inhibited, using halorhodopsin, PV+ neurons in BLA. The inhibition of PV+ neurons in BLA at P21 resulted in a significant increase of freezing in ELS animals (% time freezing: 41% ELS Controls vs. 76% ELS Halo; $P < 0.02$) to levels comparable to controls (% time freezing: 69% UHC Controls). However, it remains unclear whether ELS effects the development of mPFC inputs into BLA or whether ELS may be resulting in lowered responses to fear condition as a result of heightened activity thresholds in BLA or downstream structures. Ongoing experiments aim at answering these questions.

Disclosures: G. Manzano-Nieves: None. **K.G. Bath:** None.

Poster

726. Early-Life Stress: Perinatal

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Topic: F.04. Stress and the Brain

Support: Robert and Nancy Carney Fund for Scientific Innovation

Norman Prince Neuroscience Institute Translational Research

Title: Brain-derived neurotrophic factor: a potential driver of the accelerated neurobehavioral development induced by early-life stress

Authors: *K. B. HUNTZICKER[1], G. MANZANO-NIEVES[1], T. M. MOSS[1], K. G. BATH[2]; [1]Dept. of Neurosci., [2]Dept. of Cognitive, Linguistic and Psychological Sci., Brown Univ., Providence, RI

Abstract: Exposure to early life stress is known to increase the risk of anxiety-like and depressive-like behaviors in both human and mice models. While a great deal of research has investigated the long-term implications of early-life stress (ELS) in adulthood, its effects on development and the mechanisms driving aberrant outcomes remain largely unknown. We hypothesize that these outcomes are the consequence of stress-associated changes in the timing of neurodevelopmental events, ultimately impacting circuit structure and function. We further predict that alterations in neurodevelopment result from atypical expression of trophic factors (BDNF), which drives an acceleration in maturation. To test this hypothesis, we used a mouse model of ELS, maternal bedding restriction from postnatal days (P) 4-11. Using this manipulation, we have previously found that ELS leads to an earlier emergence of the latent period of contextual fear inhibition and earlier expression of markers of circuit maturation. Here, we collected hippocampal brain tissue from mice across early development and assessed the effects of ELS on neurotrophin expression, and found that ELS was associated with an earlier rise in BDNF as well as suppressed expression of TrkB.T1 (an endogenous dominant negative receptor for BDNF), as assessed by realtime qPCR. Using immunohistochemistry, we also observed an earlier onset of parvalbumin expressing neurons (PV+), compared with control reared mice, a process that is known to be initiated by developmental changes in BDNF levels. To test if changes in BDNF are necessary and sufficient to alter the timing of neural and behavioral development following ELS, we employed a genetic approach in which we attempted to block developmental changes in BDNF signaling following early life stress or if elevating BDNF in the absence of stress was sufficient to drive aberrant development. Specifically, we tested if the presence of the BDNF Val66Met polymorphism, a model of diminished BDNF release, blocks accelerated neurodevelopment following ELS as well as if genetic deletion of the TrkB.T1 receptor, in the absence of ELS, leads to accelerated neurodevelopment. Based upon preliminary results, BDNF is both necessary and sufficient for ELS associated changes in the timing of neurodevelopment.

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Poster

726. Early-Life Stress: Perinatal

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Topic: F.04. Stress and the Brain

Support: CONACYT number 396827

Title: Effects of maternal deprivation on anxiety-like behaviors in wistar and wistar kyoto rats

Authors: *C. VEGA BAUTISTA, C. LÓPEZ RUBALCAVA;
Neuropharm. and Exptl. Therapeut., CINVESTAV, Mexico City, Mexico

Abstract: Several studies have shown that the development of anxiety disorders in adulthood might be a consequence of chronic stress in early life. In Wistar rats it has been reported that after the exposure to maternal deprivation, animals present depression- and anxiety-like behaviors that persist into adulthood. The Wistar Kyoto (WKY) rat strain, widely used to study hypertension related diseases, has also been proposed as a good model for the study of depression- and anxiety-like behavior, because of its exacerbated responses to stressors stimulus evidenced by increased levels of ACTH and corticosterone. The objective of the present study was to compare the effects of chronic stress during early life development in adult Wistar and WKY rats, using as chronic stressor the maternal deprivation protocol. Briefly, this protocol consists in separating the dams from their pups during 3 hours daily from postnatal day (PND) 7 to 21. In control groups, the litters stayed together until the weaning day. To avoid hormonal fluctuations only male rats were chosen. The animal model used to study the anxiety like-behavior was the defensive burying test. Animals were subjected to this test at PND 90. The results showed that maternal deprivation increases anxiety-like behaviors in Wistar rats but decreased them in the WKY strain. The WKY rats showed increased general activity and exploratory behavior. Results suggest that the WKY rat strain presents a different adaptive mechanisms to chronic stress stimulus during early life experiences.

Disclosures: C. Vega Bautista: None. **C. López Rubalcava:** None.

Poster

726. Early-Life Stress: Perinatal

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Topic: F.04. Stress and the Brain

Support: Beta Beta Beta research grant to RAH

Title: Behavioral, endocrine and physiological effects of maternal separation on Sprague-Dawley dams and pups

Authors: S. L. WEINER, R. A. HEALY, *C. R. MCKITTRICK;
Drew Univ., Madison, NJ

Abstract: Stress serves an adaptive function by causing the body to react to perceived threats to homeostasis. While acute stress is largely beneficial, chronic stress can have deleterious effects through over-activation of the hypothalamic-pituitary-adrenal (HPA) axis. Previous research has demonstrated that chronic stress associated with early life traumas can have adverse effects lasting into adulthood. This study investigated the behavioral and endocrine effects of chronic maternal separation on Sprague-Dawley pups and dams. Cohort I included 4 litters of 12 pups each; pups were separated from dams once daily for 3 hours, along with the rest of their littermates, from postnatal day 7 to 14. Cohort II included 6 litters of 8 pups each; pups were separated from their dams for 4h per day from postnatal day 6 to 21 and were kept in pairs during separation. Control animals were briefly handled and returned to their dams. The effects of maternal separation (MS) on depression-like behaviors were assessed in the forced swim test (FST); anxiety-like behaviors were tested using the elevated zero maze. In addition, effects on spatial learning and memory were tested in the Morris water maze (MWM). Approximately 5 weeks after separation and weaning, juvenile pups were subject to a single episode of restraint stress novel during which tail blood was collected for measurement of baseline, stress and post-stress corticosterone levels. In Cohort I, MS pups showed decreased latency to immobility and more time spent immobile in the forced swim test (FST); they also gained less weight than their control counterparts. Maternal separation also led to a significant impairment in learning in the MWM, although there was no effect on memory. We also observed several sex differences, as females had greater locomotor activity but took longer to reach the platform in both the learning and memory trials in the MWM. Finally, preliminary studies of the dams separated from their pups indicate trends for increases in both depression- and anxiety-related behaviors. These studies indicate that maternal separation may have negative behavioral consequences for both mothers and their offspring.

Disclosures: S.L. Weiner: None. **R.A. Healy:** None. **C.R. McKittrick:** None.

Poster

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Topic: F.04. Stress and the Brain

Support: NIH grant F31 MH100779

Title: Early-life stress and reduced MET expression interact to disrupt neuronal morphology and behavior in mice

Authors: *H. HEUN-JOHNSON[1,2], P. LEVITT[1,2];
[1]USC, Los Angeles, CA; [2]Children's Hosp. Los Angeles, Los Angeles, CA

Abstract: A common hypothesis is that early adverse experiences, combined with genetic influences, increase risk of anxiety, mood, and post-traumatic stress disorders in adulthood. Previously, our lab has identified a functional single nucleotide polymorphism in the promoter region of *MET* (rs1858830 'C' allele) that has been associated with altered functional and structural brain connectivity, and increased risk of autism spectrum disorders. In mice, reduced expression of MET results in altered anxiety-like behavior and fear acquisition and memory, reduced neuronal arbor complexity and synaptogenesis, and precocious maturation of neurons based on measures of synaptic function. Here, we induced early-life stress (ELS) using limited nesting material in *wild-type* mice and mice with reduced MET protein expression ($Met^{+/-}$) in the brain. In post-pubertal mice, we analyzed neuronal morphology of pyramidal neurons in the CA1 region of the ventral hippocampus that project to the basolateral amygdala, as well as anxiety-like behaviors, social interaction, and contextual fear acquisition, memory and extinction. We observed an increased number of affected behavioral domains in $Met^{+/-}$ mice that experienced ELS, and $Met^{+/-}$ mice were more affected by ELS than *wild-type* mice in the social interaction test. Surprisingly, we observed an absence of altered dendritic arbors of CA1 neurons in $Met^{+/-}$ mice exposed to ELS, but disruption with ELS or genetic reduction of *Met* alone. The altered responsiveness to ELS in neurons with reduced expression of *Met* may be due to the precocious maturational state. These novel gene-environment interaction findings suggest that developmental perturbations may differentially impact distinct functions that may exhibit distinct vulnerabilities.

Disclosures: H. Heun-Johnson: None. **P. Levitt:** None.

Poster

726. Early-Life Stress: Perinatal

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 726.18/XX17

Topic: F.04. Stress and the Brain

Support: BAP Grant 8249 to EAD

Title: Early and recent life stress influence cortisol awakening response as a function of serotonin transporter genotype

Authors: *G. DEDEOGLU, E. A. DUMAN;
Bogazici Univ., ISTANBUL, Turkey

Abstract: Research investigating the role of early and recent life stress on cortisol response yielded conflicting results, which were exacerbated by the inclusion of serotonin transporter genotype as a moderator. In this study, we aimed to investigate the combined effect of these factors on Cortisol Awakening Response (CAR). We hypothesized a lower CAR with increased recent life stress and an interaction between early life stress and serotonin transporter genotype such that S-carriers would show an increased CAR with higher early life stress, while the opposite pattern would be seen in LL homozygotes. Participants were 92 undergraduate students (75% female) providing saliva samples 0, 30 and 45 minutes after awakening for two days. Additional saliva samples were collected for genotyping. Early life stress was assessed by the Childhood Trauma Questionnaire and recent life stress was assessed by the Perceived Stress Scale. Sleep duration and time of awakening were taken as covariates because of their potential influence on CAR. For Day 1, there was a main effect of recent life stress (F (1, 82) = 4.20, p = .044) such that those with high stress showed lower CAR than those with low stress. In addition, there was a significant interaction between serotonin transporter genotype and recent stress (F (1, 82) = 4.63, p = .034) such that S-carriers showed lower CAR independent of stress, while LL homozygotes showed a decreased CAR with higher stress. In terms of early life stress, there was a significant interaction with serotonin transporter genotype (F (1, 82) = 9.74, p = .002) such that S-carriers showed increased CAR with increased early life stress, while LL homozygotes showed the opposite pattern. Finally, there was a trend level interaction between early and recent life stress on CAR (F (1, 82) = 2.63, p = .110). For individuals with high early life stress, recent stress did not influence CAR, while for those with low early life stress, increased recent stress was associated with decreased CAR. For Day 2, we observed similar trends for all analyses. Our results suggest differential effects of early and recent life stress on CAR as a function of serotonin transporter genotype that may altogether alter individuals' vulnerability for psychological disorders like depression.

Disclosures: G. Dedeoglu: None. **E.A. Duman:** None.

Poster

726. Early-Life Stress: Perinatal

Location: Halls B-H

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Topic: F.04. Stress and the Brain

Support: MEXT/JSPS KAKENHI 25893274

Grant-in-Aid for the Cooperative Research Project from Joint Usage/Research Center (Joint Usage/Research Center for Science-Based Natural Medicine) Institute of Natural Medicine, University of Toyama in 2014

Title: Social isolation rearing influences epigenetic regulation of dorsal raphe $GABA_B$ receptor and responses to social stimulation in mice.

Authors: *R. ARAKI[1], Y. HIRAKI[1], S. NISHIDA[1], N. KURAMOTO[1], K. MATSUMOTO[2], T. YABE[1];
[1]Setsunan Univ., Hirakata, Osaka, Japan; [2]Univ. of Toyama, Sugitani, Toyama, Japan

Abstract: In rodents, social isolation rearing during childhood induces behavioral abnormalities such as hyperlocomotion, aggression and depression-like behavior, and neurochemical abnormalities such as dorsal raphe hyperexcitability and an increase in extracellular serotonin level in the prefrontal cortex. Previous studies have showed that these neurochemical abnormalities are involved in a part of these behavioral abnormalities, suggesting that dysregulation of dorsal raphe function triggers abnormal behaviors. It is known that serotonergic neurons are mainly regulated by GABAergic systems in the dorsal raphe nucleus (DRN). In this study, we examined the impact of isolation rearing on dorsal raphe GABAergic function. We also studied the involvement of isolation rearing-induced dorsal raphe GABAergic dysfunction in the abnormal behaviors. Both mRNA and protein levels of $GABA_{B1a}$ (a $GABA_B$ receptor subunit) were increased in the DRN of isolation-reared mice. DNA hypomethylation and histone H3 hyperacetylation around the transcription start site of $GABA_{B1a}$ were observed in the DRN of isolation-reared mice. Intra-DRN microinjection of 0.3 nmol phaclofen (a $GABA_B$ receptor antagonist) attenuated encounter-induced hyperactivity and aggressive behavior of isolation-reared mice. These findings indicate that an increase in dorsal raphe $GABA_{B1a}$ expression via epigenetic regulation may be associated with abnormal responses to social stimulation such as encounter-induced hyperactivity and aggressive behavior in isolation-reared mice.

Disclosures: R. Araki: None. **Y. Hiraki:** None. **S. Nishida:** None. **N. Kuramoto:** None. **K. Matsumoto:** None. **T. Yabe:** None.

Poster

726. Early-Life Stress: Perinatal

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Topic: F.04. Stress and the Brain

Support: NIMH Silvio Conte Center (P50MH094271)

Humboldt Foundation

Title: Early adversity uncouples preference formation from anxiolysis in mice

Authors: *H. S. KNOBLOCH, E. J. KIM, Z. YE, T. K. HENSCH;
MCB, Harvard Univ., Cambridge, MA

Abstract: Early adversity impacts emotions, behavior, cognitive abilities and susceptibility to psychiatric illnesses throughout life. One link may be that early life stress (ELS) alters the timing of biological critical periods for circuit maturation. Under typical rearing conditions, juvenile rats appear to be protected from exhibiting anxiety, whereas after adverse rearing, fear learning appears more mature, making stressful memories persist (Callaghan et al, 2013). Whether this is due to a shifted onset, closure or duration of a critical period remains unclear. Here, we leveraged a novel prefrontal critical period for the acquisition of preference behaviors in mice with a clearly defined window of plasticity (Yang et al, 2012). Exposure to music between postnatal day P15-25 induces a lifelong preference for that acoustic environment over the animal's innate bias for silent shelter. Acquisition of such a preference is at the same time anxiolytic, increasing open field center crossing or stress measures on an elevated platform. Adult animals can be made to acquire preference / anxiolysis if plasticity is extended or reopened by the pharmacological (HDAC inhibitors) or genetic (NgR) disruption of molecular 'brakes' on plasticity (Yang et al, 2012). Here, to study the impact of ELS on critical period timing, we examined a 'fragmented' maternal care (FC) paradigm in mice, in which reduced nesting material creates a stressful situation that disrupts mother-infant interaction during P2-9 (Ivy et al., 2008). We found that female offspring in particular exhibited heightened anxiety in adulthood. Strikingly, they failed to acquire a music preference despite exposure at various time windows after hearing onset. Instead, the anxiolytic effect of music was still observed, but only for exposure earlier than normal (P13-18). Our study reveals that preference behaviors and anxiolysis are dissociable, and that strategically timed music exposure may help to counteract some of the debilitating effects of ELS. Ongoing work is exploring the oxytocin system - which develops in an experience-dependent manner during the first postnatal weeks in rodents and underlies the formation of partner preference in voles - as a potential mechanism for critical period shifting by ELS.

Disclosures: H.S. Knobloch: None. **E.J. Kim:** None. **Z. Ye:** None. **T.K. Hensch:** None.

Poster

726. Early-Life Stress: Perinatal

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 726.21/XX20

Topic: F.04. Stress and the Brain

Support: Einhorn Family Charitable Trust

Title: Maternal potentiation of ultrasonic vocalizations is not a marker of stress

Authors: *L. M. ROSKO[1], M. ANWAR[1], J. KAIDBEY[2], A. ELLISTON[2], M. G. WELCH[1]; [1]Psychiatry, Columbia Univ. Med. Ctr., New York, NY; [2]Columbia Univ. Inst. of Nutr., New York, NY

Abstract: Our hypothesis is that nurturing reunions will shorten time required to reduce distress from a separation. In our clinical studies, the family nurture intervention repeats calming activities during mother and premature infant reunions. It is known that disruptions in mother-infant interactions decrease weight gain and impair development in human neonates. Paradigms with short periods of maternal deprivation are commonly used in preweaning rat pups to mimic disruptions in nurturing relationships. Many mammalian species, including rats, vocalize after isolation or environmental stress. Few have studied reunions. Maternal cues are deemed important for calling, as pups have higher ultrasonic vocalization (USV) rates after a separation following a brief reunion with a dam than with littermates, a phenomenon called maternal potentiation. Here, we further study the effect of separation/reunion by correlating maternal potentiation with an index of physiological stress—corticosterone (CORT) measurement. Twelve day old Sprague Dawley pups were separated overnight, and USVs were counted the next day to assess whether pups potentiated in the separation paradigm. Plasma CORT was measured in at 30, 60 or 90 minutes after separation after a dam or littermate reunion using a radioimmunoassay. Vocalizations increased an average of 62.3±9.73 for maternally reunited pups while pups reunited with littermates increased by average of 3.24±1.71. Baseline CORT in animals was 99.3±7.08 ng/mL. At 60 minutes, CORT levels were significantly higher in pups reunited with littermates compared to the dam group ($p=0.042$). Taken together, reunion type significantly changes corticosterone levels. Therefore, our data suggests that maternal potentiation is not a marker of stress since the pups that potentiated had a lower CORT response then those that did not. The lower CORT response in maternally reunited pups aligns with the idea that maternal potentiation is a pup's expression of an expectation that the dam is close enough to return upon calling. Taken together with our findings that vagotomy blocks potentiation, the low CORT response in maternally reunited pups signal the primacy of the dyadic relationship. Extrapolating to human preterm infants, our results suggest that babies who have a reunion with their mother, albeit a brief one, may be able to better cope with the stress of maternal separation.

Disclosures: **L.M. Rosko:** None. **M. Anwar:** None. **J. Kaidbey:** None. **A. Elliston:** None. **M.G. Welch:** None.

Poster

726. Early-Life Stress: Perinatal

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Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 726.22/XX21

Topic: F.04. Stress and the Brain

Support: DA029989

MD007592

Title: Effects of maternal separation on vulnerability to methamphetamine and synaptic plasticity

Authors: J. N. HAMDAN[1], S. SAUCEDO, Jr.[1], G. A. LODOZA[1], J. A. SIERRA-FONSECA[1], L. E. O'DELL[2], *K. L. GOSSELINK[3];
[1]Biol. Sci., [2]Psychology, [3]Univ. of Texas at El Paso Dept. of Biol. Sci., El Paso, TX

Abstract: Methamphetamine addiction affects roughly 1.2 million people nationally, and stress has been previously shown to affect the incidence and progression of addictive behaviors. It is not fully understood, however, which stress-induced mechanisms in the brain are involved in mediating these effects. We hypothesized that exposure to stress during early life can cause significant and long lasting changes in the brain, leading to enhanced vulnerability to methamphetamine addiction. Male Wistar rats were maternally separated (MS) from their dams for 3h/d from postnatal day (PND) 2 to PND14 and were assessed in adulthood (PND 70-80). Proteins involved in dopaminergic signaling [dopamine transporter (DAT), dopamine receptor-2 (D_2), tyrosine hydroxylase (TH)] or synaptic plasticity [post-synaptic density 95 (PSD95), NMDA receptor-1, and α-synuclein] were evaluated by Western blot in adult MS and control animals. Brain regions in which the levels of these proteins were quantified included the prefrontal cortex (PFC), hippocampus, dorsal striatum, and the nucleus accumbens (NAcc). Preliminary data have shown a significant increase in the expression of DAT in the dorsal striatum ($p=0.035$), as well as a trend toward decreasing expression of α-synuclein in the PFC ($p=0.099$) following MS. Separate groups of animals that underwent the same MS procedure were behaviorally tested for conditioned place preference (CPP) with 1 mg/kg methamphetamine, to identify changes in drug sensitivity caused by MS. Stressed animals did not demonstrate a change in preference for methamphetamine at this dose, compared to controls. Future studies will test different doses of methamphetamine to evaluate changes in preference or aversion caused by MS.

Disclosures: J.N. Hamdan: None. **S. Saucedo:** None. **G.A. Lodoza:** None. **J.A. Sierra-Fonseca:** None. **L.E. O'Dell:** None. **K.L. Gosselink:** None.

Poster

726. Early-Life Stress: Perinatal

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Topic: F.04. Stress and the Brain

Support: NARSAD Young Investigator Award

Quinnipiac University

Title: Microglial alterations after maternal separation in rodents

Authors: *L. TELISKA[1], R. ROTOLO[2], C. LITTLE[1], T. STRANGE[1], T. MEDWID[2], L. FRUEHAUF[1], A. ROSELUND[1], K. JONES[2], A. J. BETZ[1];
[1]Psychology, [2]Hlth. Sci., Quinnipiac Univ., Hamden, CT

Abstract: There is a great demand for therapeutic advancements with respect to mood disorders and a significant link between stress and major depressive disorder (MDD) exists. Maternal separation in rodents is a widely used animal model used to induce early-life stress. This model has reliably demonstrated an increased risk of depressive-like behavior later in life. Existing research also supports that individuals with MDD have learning and memory deficits. Two regions of the hippocampus, the dorsal and the ventral regions, are suggested to be functionally different while the dorsal hippocampus is involved in cognitive function and the ventral hippocampus is involved in emotion and stress. Additionally, microglia activity is associated with causing the atrophy and pathology of these brain regions and may be implicated in MDD. The proliferation and stability of microglia across the development into adulthood differs. We aimed to examine the expression of dorsal and ventral hippocampal microglia in rats that experience postnatal maternal separation during development. In the present study, Sprague Dawley male and female rat pups were separated from the dam from PND 2 to PND 14 for three hours a day. A control condition of non-separated pups was maintained. Experiment 1 examined behavioral tasks during adolescence in males and during adulthood in females. Alterations in hippocampal IBa1, CD11b and cytokine expression were observed and may provide insight as to the molecular mechanisms responsible for hallmark MDD pathogenesis and possible therapeutic remedies.

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Poster

727. Stress: Prenatal

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Topic: F.04. Stress and the Brain

Support: CONACYT 238313

CONACYT 221092

Title: Alterations related by gestational stress in glial morphology and working memory of the adulthood rats

Authors: *Y. RUVALCABA DELGADILLO[1], T. MORALES-SALACEDO[1], G. CHIPRES-TINAJERO[1], G. YAÑEZ-DELGADILLO[1], R. RAMOS-ZUÑIGA[1], A. FERIA-VELASCO[2], J. GARCÍA-ESTRADA[3], S. LUQUIN[3], F. JAUREGUI-HUERTA[3];
[1]Neurosci., [2]Univ. De Guadalajara, Guadalajara, Mexico; [3]Ctr. de Investigación Biomédica de Occidente, Guadalajara, Mexico

Abstract: El estrés crónico durante el embarazo puede afectar negativamente a las respuestas biológicas y de comportamiento en un largo plazo. Algunos de estos efectos están relacionados con los cambios estructurales en regiones específicas del cerebro. La corteza prefrontal medial (córtex prefrontal medial) es una región del cerebro que se ha relacionado con las funciones cognitivas, la memoria de trabajo y especifically atencional conjunto de la delegación de funciones. mPFC puede alterar su función y morfología de los acontecimientos estresantes durante la vida temprana. Las células gliales juegan un papel clave en la función cerebral. Los astrocitos son el tipo celular más prevalente en el sistema nervioso central, a regular neuronal micro-entorno, los circuitos de sinapsis y que se ha relacionado con el aprendizaje y la memoria. Los astrocitos también juegan un papel central en la defensa contra el daño mediante la modificación de su actividad proliferativa y morfología. Nosotros evaluamos los Efectos de un largo Plazo del estrés crónico las variables (CVS) Durante la gestación Sobre la Proliferación de astrocitos en el córtex prefrontal medial y la Memoria de Trabajo de Las Ratas Adultas Macho. Métodos: Ratas Wistar hembras expuestos were una . Durante CVS 13-19 Días de gestación . Despues del nacimiento, las Crías de Madres estresadas permanecen en las Condiciones de laboratorio Hasta cuatro meses de age Un this age se evaluaron la Memoria del Trabajo y de Atención : tareas alternancia / discrimination con laberinto de la ONU en T. despues de eso, se analizó la Proliferación de astrocitos en el córtex prefrontal medial. Resultados: Las ratas expuestas a CVS Durante el período m de gestación mostraron menor incapaz para resolver Una Memoria de Trabajo y no se observaron Diferencias: tareas y alternancia / discrimination de Atención en el Número de Células astrogliales en el córtex prefrontal medial. Conclusión: La

Exposición de un CVS Durante el embarazo producen: efectos perdurables Relacionados: tareas córtex prefrontal medial y Su Población de astrocitos.

Disclosures: **Y. Ruvalcaba Delgadillo:** None. **T. Morales-Salacedo:** None. **G. Chipres-Tinajero:** None. **G. Yañez-Delgadillo:** None. **R. Ramos-Zuñiga:** None. **A. Feria-Velasco:** None. **J. García-Estrada:** None. **S. Luquin:** None. **F. Jauregui-Huerta:** None.

Poster

727. Stress: Prenatal

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Topic: F.04. Stress and the Brain

Support: The Patterson Trust Clinical Research Award

NIH Grant KO8 MH086812-06

NIH Grant T32 NS 7421-16

Title: The role of oxidative stress in the effect of prenatal stress on the fetal brain

Authors: ***J. BITTLE**, S. J. LUSSIER, J. MICHAELSON, H. E. STEVENS;
Univ. of Iowa, Iowa City, IA

Abstract: Stress experienced by the mother during pregnancy is linked to an increased risk of psychiatric disorders in her offspring. However, the molecular mechanisms that link prenatal stress (PS) and psychiatric outcomes have yet to be elucidated. Oxidative stress has been shown to be a mediator of the impact of multiple environmental exposures, making it a prime candidate for mediating the effects of in utero environmental changes like PS. The balance between oxidative and reductive processes is critical during rapid periods of cell growth and differentiation, so small changes in the level of reactive oxygen species (ROS) during embryonic brain development could have significant effects. We used two approaches to investigate oxidative stress as a mechanism for PS, both utilizing a mouse model of repetitive restraint stress starting on embryonic day 12 (E12). First, we examined changes in gene expression in E13 brain after PS by Illumina microarray and qPCR validation. Second, we evaluated the impact of antioxidant administration during PS on GABAergic migration delays. After correcting for multiple comparisons, we found 5177 genes significantly altered in E13 ventral forebrain after one day of PS. Panther analysis of these genes revealed that 38 pathways were enriched for differentially expressed genes-- the oxidative stress pathway was the third most significant pathway changed ($p= 0.0000027$). In addition, four of the six genes annotated with “antioxidant

activity" by Panther were differentially expressed, including sestrin 1, sestrin 3, glutathione peroxidase, and catalase. 421 genes were found to be differentially regulated by PS in males versus females. Two of the top 15 genes from this analysis were within pathways contributing to ROS-- *Aifm1* and *Idh2*. When examined by qPCR, both *Aifm1* and *Idh2* were significantly changed only by PS, with no effect of sex. These results reveal that genes in oxidative stress and antioxidant pathways are a significant component of PS changes in the fetal brain. In the second experiment, N-acetylcysteine (NAC), a common antioxidant supplement, was administered in drinking water to pregnant dams starting at E0 with half of dams undergoing PS at E12 and half maintained as controls. We have shown previously that PS delays GABAergic progenitor migration. Pilot data showed maternal NAC administration normalized GABAergic migration at E13, but not E14, suggesting that oxidative stress may only play a role in acute, but not prolonged, changes after PS. Oxidative stress may play a significant role in the elusive mechanisms by which persistent cellular changes are induced in the developing brain by maternal PS.

Disclosures: J. Bittle: None. **S.J. Lussier:** None. **J. Michaelson:** None. **H.E. Stevens:** None.

Poster

727. Stress: Prenatal

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 727.03/YY3

Topic: F.04. Stress and the Brain

Title: Single dose ketamine injection does not alter behavioral parameters in rats exposed to both prenatal and chronic unpredictable mild stress

Authors: *E. ULUPINAR[1], E. POLAT CORUMLU[2], O. AYDIN[2], E. GULHAN AYDIN[3]; [2]INTERDISCIPLINARY NEUROSCIENCE DEPARTMENT, [3]DEPARTMENT OF PHARMACOLOGY, [1]Eskisehir Osmangazi Univ., Eskisehir, Turkey

Abstract: In recent clinical trails, NMDA receptor antagonist ketamine has been shown to act as an antidepressant, due to its rapid action and efficacy on treatment-resistant patients. Antidepressant effects of a single dose of ketamine might start within hours and sustain for up to 2 weeks. Early life stress can cause long-term behavioural and morphological alterations in the central nervous system. Likewise, chronic unpredictable stress can cause depressive-like effects. In experimental models, we have previously demonstrated that exposure to either prenatal or chronic unpredictable stress paradigms can induce depressive-like behavioral deficits, as well as morphological alterations in the rat brains. The aim of this study was to investigate the behavioral effects of single dose ketamine injection in rats exposed to combination of both

prenatal and chronic unpredictable mild stress, in a gender dependent manner. Pregnant rats were exposed to immobilization stress during the last week of their gestation for 3 hours a day. Then, beginning from postnatal day 95, a random pattern of unpredictable mild stressors was applied daily, for 28 days. At the end of stress period, one group of animals received a single dose (10mg/kg) of intraperitoneal ketamine injection, whereas the other group received same amount of saline injections. Next day, behavioral responses of animals were evaluated in the forced swim, sucrose preference, open field and suspension tests. In these tests, no significant differences were observed between groups. Two-way ANOVA results showed that ketamine injections cause similar effects on both female and male animals. Although these results are in accordance with our previous findings in animals exposed to chronic unpredictable stress, it displays some controversies with the literature. Therefore, antidepressant efficacy of ketamine needs to be explored in different animal models of depression caused by various stressors. Future studies are underway to compare gender-dependent morphological and molecular effects of single dose ketamine injections.

Disclosures: **E. Ulupinar:** None. **E. Polat corumlu:** None. **O. Aydin:** None. **E. Gulhan aydin:** None.

Poster

727. Stress: Prenatal

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Program#/Poster#: 727.04/YY4

Topic: F.04. Stress and the Brain

Support: Bucknell University Funding

Title: Reductions in hippocampal AMPA receptor and NMDA receptor subunit expression are associated with impaired learning in adult male rats following prenatal exposure to Dexamethasone

Authors: C. R. DODSON, *K. C. PAGE;
Bucknell Univ., Lewisburg, PA

Abstract: Antenatal glucocorticoids (GC) have been used clinically to enhance lung maturation in the fetus at risk for preterm birth. However, exogenous Dexamethasone (Dex) has been associated with a disturbance of the normal relationship between components mediating glucocorticoid action in the hippocampus. It is possible that these agents may increase the risk for physical, neurological, and behavioral alterations. Perturbations in GC programming in the fetal brain could affect neuritic outgrowth, synaptic activity and cell signaling through

glutamatergic and serotonergic circuits. We hypothesized that exposure to excess glucocorticoid during fetal development exerts a deleterious effect on expression of hippocampal genes mediating synaptic function and behavior in adult male rats. Pregnant rat dams were injected once daily with 150ug/kg of Dex (sc) from gestation-day 14 through 19. Control dams were injected with vehicle. At 120 days, male offspring were tested for learning and memory function using the Morris Water Maze. At 150 days, whole hippocampi were rapidly dissected and frozen using liquid nitrogen. Gene Expression was measured using real-time PCR. Using the Morris Water Maze protocol, we found that the Dex-exposed males spent more time trying to find the platform even after 5 days of training (longer latency, $p = 0.05$). Expression of key genes known to mediate this behavior was also perturbed as follows: In the Dex-exposed animals, a significant reduction was detected in AMPA receptor subunits GluR1 and GluR2 ($p =0.002$ and $p<0.02$, respectively) as well as the NMDA receptor subunits NR2A and NR2B ($p<0.02$ and $p =0.002$, respectively). The mRNA expression for the NR1 subunit, a constitutive subunit for NMDA receptors, was not significantly altered. Interestingly, the mRNA expression for serotonin receptors 5HT1A, 5HT1B and 5HT2A was significantly increased in the Dex-exposed male offspring, perhaps due to a compensatory response. Synaptotagmin and SNAP-25 mRNA expression was not significantly altered. These data support the observation that gene-environment interactions during critical periods of neurogenesis lead to developmental changes that persist into adult life. More specifically, prenatal exposure to synthetic glucocorticoid at key points of brain development disturbs glutamatergic and serotonergic function which may be associated with learning and memory deficits in adult hood.

Disclosures: C.R. Dodson: None. **K.C. Page:** None.

Poster

727. Stress: Prenatal

Location: Halls B-H

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Program#/Poster#: 727.05/YY5

Topic: F.04. Stress and the Brain

Support: CONACYT, Registro 001482 clave DAFCYT-2003IDPTNNN0020

Title: Learning and memory in prenatally stressed rats and its relation with hippocampal serotonin and norepinephrine release. Learning and memory in prenatally stressed rats and its relation with hippocampal serotonin and norepinephrine release.

Authors: *D. MÉNDEZ-GUERRERO[1], F. J. JIMÉNEZ-VÁSQUEZ[1], C. RUBIO OSORNIO[2], L. L. ROCHA ARRIETA[3], S. RETANA-MARQUEZ[4];
[1]Grad. Exptl. Biol. Univ. Autónoma, Ciudad DE Mexico, Mexico; [2]Departament Neurophysiol.,

Inst. Natl. of Neurol. and Neurosurg., Ciudad DE Mexico, Mexico; [3]Departament Pharmacol., Ctr. for Res. and Advanced Studies of the Inst. Natl. Polytechnic, Ciudad DE Mexico, Mexico; [4]Dept. of Reproductive Biol., Univ. Autonoma Metropolitana, Ciudad DE Mexico, Mexico

Abstract: Stress causes deleterious effects in health and may occur at different stages of life, even in the gestational period. Prenatal stress (PS) is capable of disrupting hippocampal physiology through maternal glucocorticoids (GC), decreasing glucocorticoid receptors in that brain structure, causing alterations in learning and memory in the adult. Thus, the aim of this study was to evaluate learning and memory in prenatally stressed rats and whether these changes are associated with alterations in serotonin and noradrenaline release in the dorsal hippocampus. Pregnant females were assigned to control or stress groups. Spatial learning and memory were evaluated with the Morris Water Maze (MWM) in 2 ½ months old offspring. Cannulas were implanted in the dorsal hippocampus of other adult offspring. The dialysates were collected each hour before and after administration of high doses of potassium. Neurotransmitters were detected by HPLC-ED. The results showed greater escape latencies as well as less time in the platform quadrant and platform-site crossovers, compared to the control rats. Additionally, basal serotonin and norepinephrine release were greater in the PS group than in the control. However neurotransmitter release decreased after high doses of potassium, suggesting that serotonin and norepinephrine neurotransmission is disrupted by prenatal stress.

Disclosures: D. Méndez-Guerrero: None. **F.J. Jiménez-Vásquez:** None. **C. Rubio Osornio:** None. **L.L. Rocha Arrieta:** None. **S. Retana-Marquez:** None.

Poster

727. Stress: Prenatal

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Topic: F.04. Stress and the Brain

Support: CONACYT, registro 001482clave DAFCYT-2003IDPTNNN0020

Title: Prenatal stress induced depressive-like behaviour associated with serotonin release in the ventral hippocampus

Authors: *F. J. JIMENEZ-VASQUEZ[1], D. MÉNDEZ-GUERRERO[2], C. RUBIO OSORNIO[3], L. L. ROCHA ARRIETA[4], S. RETANA-MÁRQUEZ[5];
[1]Grad. Exptl. Biol. Univ. Autónoma, Ciudad DE Mexico, Mexico; [2]Grad. Exptl. Biol. Univ. Autónoma Metropolitana-Iztapalapa, Ciudad DE Mexico, Mexico; [3]Inst. Natl. of Neurol. and Neurosurg., Ciudad de Mexico, Mexico; [4]Ctr. for Res. and Advanced Studies of the Inst. Natl.

Polytechnic, Ciudad de Mexico, Mexico; [5]Dept. of Reproductive Biol., Univ. Autónoma Metropolitana-Iztapalapa, Ciudad de Mexico, Mexico

Abstract: Major depressive disorder or major depression is a common disorder that affects people worldwide and combines symptoms at psychological, physiological, and behavioural levels. This disorder is predicted to be the second cause of disability in 2020. Animal models of major depression caused by stress are relevant to the better understanding the aetiology of depression. Thus, the aim of this work was to evaluate whether prenatal stress induces depressive-like behavior and if this behaviour is related to changes in serotonin content and release in ventral hippocampus of the adult rats. Pregnant female Wistar rats weighing 250 grams were randomly assigned to the control group or stress by immersion in cold water from day 15 to 21 of pregnancy. At 21 days of age, pups were sexed and males were separated. Depressive-like behaviour was assessed by forced-swim tasks and sucrose intake test in male offspring at 8–12 weeks of age. Rats displaying depressive-like behaviour were selected and cannulas were stereotactically implanted into the right ventral hippocampus. The dialysates were collected and basal serotonin release was evaluated HPLC-ED. The results indicate that prenatal stress caused depressive-like behaviour, with increased immobility in the Porsolt test, as well as decreased sucrose intake (considered as anhedonia) in adult male offspring. Serotonin release was higher in prenatally stressed males. Administration of high doses of potassium did not stimulate the neurotransmitter system. These findings suggest that prenatal stress is able to induce depressive-like behaviour in adult male offspring and this could be related to disruption in serotonin release in the ventral hippocampus.

Disclosures: F.J. Jimenez-Vasquez: None. **D. Méndez-Guerrero:** None. **C. Rubio Osornio:** None. **L.L. Rocha Arrieta:** None. **S. Retana-Márquez:** None.

Poster

727. Stress: Prenatal

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NARSADYoung Investigator Award

Title: Changes in cortical GABAergic maturation after prenatal stress and the role of oxidative stress

Authors: P. W. ABBOTT, S. J. LUSSIER, *H. E. STEVENS;
Univ. of Iowa, Iowa City, IA

Abstract: Objective: Our lab seeks to understand the mechanisms by which early developmental events increase the risk of psychiatric illness. The purpose of this ongoing study is to determine further how prenatal stress (PS), specifically, perturbs cortical maturation.
Background: We have found previously that PS delays GABAergic progenitor migration and postnatal pruning of cortical populations during development in mice. These delays may contribute to disruptions of cortical inhibitory circuitry theorized to be a component of psychiatric pathology. How PS may affect other aspects of cortical maturation is not clear. The formation of extracellular perineuronal nets (PNNs) around GABAergic neurons has been implicated in regulating developmental plasticity. PNNs, their maturation, and their "protection" of GABAergic neurons may be influenced by oxidative stress, a possible mechanism by which PS influences the brain. To examine these issues, we measured multiple aspects of cortical GABAergic neuron maturation in a mouse model of PS, targeting one possible mechanism, oxidative stress, through administration of a known antioxidant N-acetylcysteine (NAC).
Methods: In a cohort of pregnant female CD1 mice, half underwent PS which consisted of 45 minutes within a restraint tube three times a day starting at embryonic day 12 (E12), seven days prior to parturition. In the perinatal period, mothers and pups were given either normal drinking water or NAC (2 mg/mL) in drinking water. Offspring were tested on behavioral tasks at weaning age and in adulthood. By using stereology with immunostaining, we examined GABAergic neurons, their activation (by cFOS) and surrounding PNNs in medial frontal cortex (mFC) at postnatal day 24 (P24) and in adulthood.
Results: PS resulted in reduced sociability in adulthood as well as increased anxiety and motor inhibition at P24 and in adulthood. Pilot data suggest amelioration of some of these behavioral changes with early postnatal NAC administration. We have previously found that PS increases mFC GABAergic cell number at P24 but not in adulthood. We have new data suggesting that PS decreases PNN+ GABAergic cells at P24 and cFOS+ GABAergic cells at P24. Findings from mFC at P24 suggest that early NAC exposure normalized GABAergic population numbers and may show some amelioration of PNN+GABAergic proportions.
Conclusion: This ongoing study provides initial evidence that PS may affect multiple aspects of GABAergic interneuron maturation in mFC, coincident with behavioral abnormalities. Postnatal enhancement of antioxidant systems may affect some but not all of these maturational changes after PS.

Disclosures: P.W. Abbott: None. **S.J. Lussier:** None. **H.E. Stevens:** None.

Poster

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Topic: F.04. Stress and the Brain

Support: CIHR

Title: Late prenatal dexamethasone exposure and induced fetal programming of behavioural deficiencies

Authors: *C. LALONDE[1], J. GRANDBOIS[2], K. ZIMANY[2], S. KHURANA[5], S. THARMALINGAM[5], T. C. TAI[2,5,3,4];
[1]Biomolecular Sci., Laurentian Univ., North Bay, ON, Canada; [2]Biomolecular Sci., [3]Chem. and Biochem, [4]Biol., Laurentian Univ., Sudbury, ON, Canada; [5]Med. Sci. Div., Northern Ontario Sch. of Med., Sudbury, ON, Canada

Abstract: Prenatal exposure to stress during critical periods of fetal growth and development has serious behavioural and health implications for the offspring. The effects of prenatal exposure to stress are poorly understood; evidence is accumulating that these vulnerabilities span future generations. To investigate the potential behavioural deficits on multiple generations of offspring, pregnant Wistar-Kyoto rats were exposed to one of two treatment regimes during the equivalency of the third trimester (beginning on gestational day 15): daily IP injections until birth of 1) a synthetic glucocorticoid, dexamethasone (DEX; 100µg/kg/day) or 2) vehicle (4% ethanol with 0.9% saline). A third group received only physical manipulation to serve as a naive control. The offspring from each litter (F1 generation) were allowed to mature and were tested during week 17 for indications of anxiety and depression through use of the Elevated Plus Maze (EPM) and the Porsolt Swim Test (PST) respectively. Randomly selected males and females were bred with new naive animals and the following generation of offspring (F2) were allowed to mature and underwent the same behavioural testing. Further, a third generation (F3) was generated using the animals from the F2 generation. Neural tissue from each generation was collected and stored at -80°C for histological and immunohistochemical (IHC) processing to investigate deficits in neurogenesis and changes in glucocorticoid receptor expression. Preliminary results indicate exposure to prenatal DEX predisposes adult offspring to exhibit depressive-like behaviours that spans several generations. The PST results for the F1 generation primarily show a significant effect on the first day of testing for total time spent immobile during the procedure ($\chi^2 = 10.002$, $p < 0.001$), and a similar trend with other generations. EPM results from the F1 generation show a main effect ($Z = -1.952$, $p < 0.05$), where females spend significantly more time in open arms than males. The F2 and F3 generations exhibited the same sex effect with females spending more time in open arms than males ($p < 0.05$). The F2

generation also showed a significance between groups ($F(5, 44) = 2.62$, $p = 0.037$), whereby naive males and females spent significantly more time in open arms than DEX F1 male offspring. Altogether, these results suggest that late prenatal exposure to stress causes long-term behavioural changes in the offspring that is also apparent in subsequent generations.

Disclosures: **C. Lalonde:** None. **J. Grandbois:** None. **K. Zimany:** None. **S. Khurana:** None. **S. Tharmalingam:** None. **T.C. Tai:** None.

Poster

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Topic: F.04. Stress and the Brain

Support: Arizona Biomedical Research Commission 14-082990

Title: Prenatal dexamethasone exposure alters the postnatal leptin surge in rats

Authors: *B. HAMMOND, M. K. THOMPSON, L. MADHAVPEDDI, T. M. HALE, R. J. HANDA;
Univ. of Arizona, Phoenix, AZ

Abstract: Overexposure to glucocorticoids during gestation increases the risk in adulthood for developing disease such as metabolic syndrome. However, little is currently known about the mechanisms underlying the fetal programming of adult disease by glucocorticoids. In rodents, a potential target for the programming of metabolic syndrome is the development of hypothalamic circuity that controls food intake, metabolism and adiposity which is largely dependent on a surge of leptin during the second week of postnatal development. This study investigated the effects of maternal exposure to synthetic glucocorticoids (sGCs) on the surge of leptin during early postnatal development as a candidate hormone for the fetal programming of adult disease. In this study, time-pregnant dams were administered 0.1 and 0.4 mg/kg dexamethasone (Dex) daily during days 18-21 of gestation. Blood was collected from offspring on postnatal days (PD) 0, 7, 10, and 13 and plasma was then assayed for leptin levels using an enzyme-linked immunosorbent assay. Prenatal Dex treated animals were significantly smaller at birth and as we have previously reported, when allowed to grow to adulthood, these animals exhibit some signs of metabolic syndrome such as macrovesicular hepatic steatosis as detected by Oil Red O staining in liver. During neonatal life, there was a significant elevation in plasma leptin levels which peaked in vehicle treated animals at PD 10 (8.2 ± 1.1 ng/ml) and 13 (10.2 ± 1.9 ng/ml). Moreover, 0.1 mg/kg Dex significantly reduced plasma leptin at PD 10 (4.0 ± 0.3 ng/ml) and 13

(6.7 ± 0.7 ng/ml), while 0.4 mg/kg Dex significantly reduced plasma leptin at PD 13 (6.9 ± 1.3 ng/ml) as compared to vehicle-treated controls. We are currently determining the consequences of alterations in the leptin surge during early postnatal development by prenatal Dex treatment. An altered leptin surge could dramatically alter the development of hypothalamic circuity controlling food intake and adiposity, thereby shedding light on a potential mechanism by which maternal exposure to sGCs programs adult metabolic syndrome.

Disclosures: B. Hammond: None. **M.K. Thompson:** None. **L. Madhavpeddi:** None. **T.M. Hale:** None. **R.J. Handa:** None.

Poster

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Title: Effect of prenatal restraint stress in a DN-DISC1 mouse paradigm for schizophrenia

Authors: *S. N. REID[1], K. S. JONES[2], M. C. GONDRE-LEWIS[1];
[1]Dept. of Anatomy, Lab. for Neurodevelopment, Howard Univ. Col. of Med., Washington, DC;
[2]Dept. of Biol., Howard Univ., Washington, DC

Abstract: Schizophrenia is a devastating disorder with a worldwide prevalence of 0.5-1.2% (Kessler et. al., 2005), generally presenting in late adolescence and early adulthood (Kessler et al., 2007). Previously, a majority of gene-environment studies involving dominant-negative Disrupted-in-Schizophrenia-1 (DN-DISC1) have focused on prenatal infection and immune activation (Abazyan et al., 2010; Hida et al., 2013; Lipina et al., 2013). It is unclear how the interplay between the presence of a susceptibility gene and exposure to environmental stressors, in particular prenatal stress, increase the risk of neuropsychiatric disorders, such as schizophrenia. Putative vulnerability genes, such as DISC1, when exposed to excessive trauma, may act in concert and produce disruptions in neurodevelopmental events.
Here, we examine alterations in reciprocal circuits of the amygdala and hippocampus in a DN-

DISC1paradigm submitted to prenatal restraint stress. Biochemical and immunohistochemical data suggest expression of DN-DISC1 appears to reduce expression of the calcium binding protein, parvalbumin, and glutamate receptor subunits, GluN1 and GluA1, in the hippocampus and amygdala. These data imply alterations in the amygdalo-hippocampal circuit that may be central to the pathophysiology of fear circuits in schizophrenia and may be severely altered when the genetic predisposition is combined with early life stress.
Since neurodevelopmental landmarks and the molecular mechanisms that govern fear memory are highly implicated in the etiology of schizophrenia, the impact of DN-DISC1 expression combined with prenatal stress exposure in fear circuitry was examined using a battery of behavioral tests and biochemical assays. Fear conditioning, specifically contextual and cued conditioning, suggest potential effects between the presence of expressed DN-DISC1 and *in utero* stress via restraint of the pregnant dam.
While one particular gene may not cause schizophrenia, characterizing the gene-environment interaction in this disorder will help to further our understanding of its etiology and provide insight into the risk of its development possibly creating interventions to help target or alleviate its symptoms. As mentioned previously, there is an abundance of evidence to suggest that the GABA system is associated with dysfunction in cognitive processing, especially fear memory. By defining the GABA system within fear circuitry after prenatal stress exposure in the presence of expressed DN-DISC1, we may be able to find new evidence to target the developmental time points significant to the onset of schizophrenia.

Disclosures: **S.N. Reid:** None. **K.S. Jones:** None. **M.C. Gondre-Lewis:** None.

Poster

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Topic: F.04. Stress and the Brain

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Title: Effects of birth mode on developmental cell death in the mouse brain

Authors: *A. CASTILLO-RUIZ, M. MOSLEY, N. G. FORGER;
Georgia State Univ., Atlanta, GA

Abstract: Birth involves dramatic changes in a newborn's environment and the processes associated with birth prepare key peripheral organs for the transition to postnatal life. However, little is known about how birth influences the brain and behavior. We previously found that birth mode (Vaginal "V" vs Cesarean "C") may alter neurobehavioral development. In this study, we further investigated the effects of birth on brain development by focusing on cell death, a key feature of nervous system development. There is increased cell death across many brain regions around the time of birth in mice, but whether birth induces cell death, and whether mode of birth influences cell death has not been addressed. We manipulated birth mode in timed-pregnant C57BL/6 mice and collected the brains of offspring *in utero* at embryonic day (E)18.5 and E19 and *ex utero* at postnatal day (P)0 (3h after birth), P1, P3, and P23 for the histochemical detection of activated caspase-3, a cell death marker. We found that C born mice had higher cell death perinatally across many brain regions, including hypothalamic, epithalamic, hippocampal and midbrain subregions. We are currently assessing whether these differential patterns of cell death induce long-lasting effects on regional cell number and volume. We are also processing the brains for the histochemical detection of microglia, the brain's resident immune cells. Uterine inflammation triggers labor, and parturition activates the peripheral immune system of the offspring. Our previous results show that birth is associated with dynamic changes in pro- and anti-inflammatory cytokines in the periphery. Whether the immune activation at birth extends to the brain is not known and will be investigated here. Together our results suggest that birth may be an important event for brain development and deviations from the natural mode of birth may alter brain and behavioral development.

Disclosures: **A. Castillo-Ruiz:** None. **M. Mosley:** None. **N.G. Forger:** None.

Poster

727. Stress: Prenatal

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Topic: F.04. Stress and the Brain

Support: Brain Health Institute, Emory University

Department of Psychiatry and Behavioral Sciences, Emory University School of Medicine

Yerkes National Primate Research Center, Emory University

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Title: Influence of *In utero* stress on fear memory consolidation in adulthood.

Authors: A. E. EASTON, *B. G. DIAS;
Emory Univ., Atlanta, GA

Abstract: Early life stress has been recognized to have profound and long-lasting effects in humans, catalyzing the development of neuropsychiatric disorders such as depression, anxiety disorder and Post-Traumatic Stress Disorder (PTSD). To understand how early life stress exerts these effects, diverse rodent models have shown varying but significant effects on physiological and behavioral endpoints in adulthood. However, little research has been done exploring the effects of *in utero stress* on fear learning in adults: an influence that may have direct consequences for the development of PTSD. We explored the relationship between *in utero* stress and the formation of fear memories using classical conditioning. Pregnant female C57/Bl6J mice were administered five un-signaled mild foot-shocks, once per day for 3 days, causing *in utero* stress to the gestating offspring on embryonic days 17-19 post-fertilization. In adulthood, F1 males were first tested in an Elevated Plus Maze to test anxiety-like behavior, and no differences were noted between non-stressed F1 offspring (F1-Control) and F1 animals that experienced *in utero* stress (F1-Stressed). Animals then underwent auditory fear conditioning where neutral tone presentations co-terminated with mild foot-shocks. Using freezing as a measure of learning and memory, the acquisition of fear was examined during training, and the consolidation of fear memory was examined one day later by presenting the same tone in a different context. F1-Control and F1-Stressed males did not acquire fear any differently from each other during training. In contrast, F1-Stressed males demonstrated lower levels of freezing upon tone presentations on the second day compared to F1-Control males suggesting that *in utero* stress impairs the consolidation of fear memory. These data suggest that stress experienced *in utero* results in deficits in the consolidation of emotional memories, which may contribute to the development of neuropsychiatric disorders like PTSD.

Disclosures: A.E. Easton: None. **B.G. Dias:** None.

Poster

727. Stress: Prenatal

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Topic: F.04. Stress and the Brain

Support: CONICET- PIP 0249

Title: Gestational stress consequences on prepubertal male offspring behavior are possibly mediated by epigenetic changes in the hippocampus

Authors: *M. E. PALLARÉS[1], M. C. MONTELEONE[2], V. PASTOR[1], A. ALZAMENDI[3], S. OLSZEVICKI[1], M. A. BROCCO[2], M. C. ANTONELLI[1];
[1]Inst. De Biologia Celular Y Neurociencia, CABA, Argentina; [2]Inst. de Investigaciones Biotecnológicas de la Univ. de San Martín (IIB- UNSAM), Buenos Aires, Argentina; [3]Inst. Multidisciplinario de Biología Celular (IMBICE-CONICET-CICPBA), La Plata- Buenos Aires, Argentina

Abstract: Chronic stress is a risk factor in the development of depression and anxiety disorders. However, variability in the stress response to chronic stress depends on the individual genetic and epigenetic background, and also on the neonatal programming of the stress response system development. Maternal stress during gestation was reported to affect fetus developing brain morphology and adult offspring behavior, by inducing changes in gene and protein expression. We have previously demonstrated that prenatal stress (PS) induces persistent changes in the expression of plasticity-related genes such as *gpm6a* and *bdnf* in the male offspring hippocampus at adulthood. Here we evaluated gestational stress consequences on dams and on prepubertal male offspring behavior and stress response. Stressed dams showed lower body weight gain in comparison with unstressed control (C) rats, despite finding similar food and water intake between both experimental groups. At post-weaning stages stressed dams spent more time immobile in the *open field* and in the *Porsolt test*, pointing out that chronic stress during pregnancy increased depression-like behavior in the rat. Conversely, PS male offspring displayed a higher ratio in the open arms entries in an elevated plus maze, indicating a reduced anxiety-like behavior than C counterparts, and they also showed a reduced depression-like behavior (*Porsolt test*). Corticosterone levels did not differ between groups after an acute stress or 2h later. Overall, our findings indicate that although stress during pregnancy negatively affected the mother behavior, offspring did not show such effects. PS offspring seemed to perform better in the behavioral tests than C rats. Thus, we propose that exposure to an adverse intrauterine environment may provide individuals useful skills to deal afterwards with harsh external conditions. This "adaptive developmental plasticity" requires of persistent changes in gene expression. Since PS modulates the expression of the plasticity-related genes *gpm6a* and *bdnf* by changes in their methylation pattern, we propose that the methylation machinery (*dnmts, methyl binding proteins, tets enzymes*) could regulate the gene expression underlying the offspring behavior.

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Poster

728. Early-Life Stress: Adolescence

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Topic: F.04. Stress and the Brain

Support: NIH Grant NR014886

Title: Chronic adolescent stress alters the adult rat transcriptome in a sex specific manner

Authors: *G. N. NEIGH[1], S. A. ROWSON[2], M. BEKHBAT[2], S. D. KELLY[2], Q. ZHAOHUI[2]; [1]Anat. & Neurobio., Virginia Commonwealth Univ., Richmond, VA; [2]Emory Univ., Atlanta, GA

Abstract: Women are more likely to develop depression and other stress-related disorders than men. Animal models have proven to be useful in studying this sex effect. Adult rats have been found to exhibit behavioral and molecular changes following exposure to chronic adolescent stress (CAS). While the behavioral alterations persist to adulthood, whether the molecular changes persist is unknown. We assessed the hippocampal transcriptome at baseline and in response to an acute stressor in adult rats with and without a background of chronic adolescent stress to assess whether molecular changes continue into adulthood. Male and female Wistar rats were exposed to a mixed-modality stress paradigm on PND 38-49 or were pair-housed controls. The stress paradigm consisted of isolation, restraint, and social defeat. On PND 94, tissue was collected from six rats per group at baseline or 30-minutes following a forced-swim acute stressor. Total RNA extracts from hippocampal tissue were used for RNA sequencing. Paired comparisons between non-stressed and CAS groups were assessed using EdgeR. Metacore was used to assess pathway enrichment. Male and female rats exposed to CAS exhibited altered patterns of differentially expressed genes at baseline and in response to an acute forced swim stressor. 63 unique genes were differentially expressed in CAS females compared to non-stressed females following an acute stressor, 11 were differentially expressed in males, and 24 genes were altered in both males and females. At baseline, 47 genes were differentially expressed in males exposed to CAS compared to non-stressed controls. No genes were altered in females at baseline. This study provides novel evidence that changes to the transcriptome following CAS are sex-dependent. These data also establish that exposure to adolescent stress results in long-term changes to hippocampal gene expression that are preserved even months removed from stress.

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Poster

728. Early-Life Stress: Adolescence

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NIDA R15 DA035478

Title: Increased function of D_2 autoreceptors contributes to reduced prefrontal cortex dopamine activity following adolescent social defeat

Authors: *M. A. WEBER, G. L. FORSTER, M. J. WATT;
Div. of Basic Biomed. Sci., Univ. of South Dakota, Vermillion, SD

Abstract: Greater incidences of psychiatric disorders in early adulthood have been linked to bullying victimization during adolescence. These disorders are characterized by impaired executive function, suggesting stress-induced disruption of the developing prefrontal cortex (PFC) dopamine (DA) system. Rats exposed to repeated social defeat in adolescence, as a model of teenage bullying, display decreased medial PFC (mPFC) DA activity in early adulthood, along with changes in mPFC DA-mediated behaviors. We hypothesize that the reduction in adult mPFC DA results from over-activation of mechanisms for coping with adolescent stress-evoked increases in DA release. In support of this, we have shown that adolescent defeat increases DA transporter-mediated clearance of DA in the adult mPFC. In addition, pharmacological activation of mPFC DA D_2 autoreceptors (D_2AR) in non-defeated adolescent rats decreases adult mPFC DA activity, mimicking the effect of adolescent defeat. Conversely, pharmacological blockade of mPFC D_2ARs during adolescent defeat prevents the later mPFC DA hypofunction. Here, we investigated whether D_2AR function in the adult mPFC is increased following adolescent defeat to further contribute to decreases in DA availability. Adolescent male rats experienced either repeated social defeat or control (novel cage exposure) from postnatal day (P)35-39. In early adulthood (P56), subjects underwent non-recovery stereotaxic surgery, with a unilateral stearate-graphite paste recording electrode implanted directly into the infralimbic (IL) region of the mPFC to measure extracellular DA using *in vivo* chronoamperometry. A stainless-steel guide cannula implanted immediately adjacent to the recording electrode allowed for infusion of vehicle (aCSF) or the D_2 receptor agonist quinpirole (3 nM/5 μL aCSF), with a decrease in extracellular DA after quinpirole infusion indicating preferential activation of D_2ARs. Preliminary data indicate that this dose of quinpirole induces a greater decrease in extracellular DA in the IL-mPFC of previously defeated rats compared to controls, suggesting that the adult mPFC DA hypofunction resulting from adolescent defeat may partly be caused by increased D_2AR function and subsequently enhanced inhibition of DA release. Such increased mPFC

D_2AR function may represent carry over of an initially adaptive mechanism for counteracting stress-evoked increases in DA release. However, its persistence into early adulthood in the absence of further stress would contribute to a lowering of mPFC DA tone, with negative implications for DA-mediated executive function.

Disclosures: **M.A. Weber:** None. **G.L. Forster:** None. **M.J. Watt:** None.

Poster

728. Early-Life Stress: Adolescence

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Topic: F.04. Stress and the Brain

Support: NIDA R15 DA035478

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NIDA R25 DA033674

Title: Disruptions to adolescent cortical dopamine activity promote seeking of amphetamine-associated cues in adulthood

Authors: ***S. ENGEL**, R. T. PAULSEN, M. A. WEBER, M. J. GERLACH, A. D. KOCH, G. L. FORSTER, M. J. WATT;
Basic Biomed. Sci., Univ. of South Dakota, Vermillion, SD

Abstract: Victims of teenage bullying show increased psychiatric disorders in adulthood, including substance abuse. This may result from stress-induced disruption of the developing adolescent brain, which undergoes dynamic reorganization in areas related to impulse control, such as the prefrontal cortex (PFC) dopamine (DA) system. This scenario can be studied using a rodent adolescent social defeat paradigm, a model of teenage bullying, which we have shown enhances seeking of amphetamine-associated cues in adulthood. Adolescent defeat also reduces adult medial PFC (mPFC) DA activity, and pharmacological depletion of mPFC DA is known to increase psychostimulant responses. However, a causal link between decreased adult mPFC DA resulting from perturbations in adolescence, such as those caused by social stress, and later increases in amphetamine responding has yet to be established. Here, we tested this by pharmacologically manipulating mPFC DA activity in non-defeated adolescent males to mimic the effects of adolescent defeat, then determined if this increased adult drug seeking. Adolescent male rats received daily bilateral infusions of either vehicle (aCSF) or the DA D_2 receptor agonist quinpirole (100 ng/μL in 0.3 μL) into the mPFC from postnatal day (P) 35 – 39. This

protocol mimics the adult mPFC DA hypofunction caused by adolescent defeat, presumably by preferentially activating presynaptic D_2 autoreceptors to inhibit DA release and synthesis. Upon reaching early adulthood (>P56), conditioned place preference (CPP) was used to assess drug cue seeking, with animals receiving either saline or amphetamine (1.0 mg/kg ip.). At P63, brain tissue was collected by rapid decapitation and then sectioned to confirm cannula placement, with efficacy of drug infusions in reducing mPFC DA determined using HPLC. Consistent with our hypothesis, adult rats that received repeated quinpirole mPFC infusions in adolescence exhibited decreased mPFC DA content and correspondingly magnified amphetamine CPP compared to vehicle-infused subjects. Findings suggest that over-activation of D_2 autoreceptors in the adolescent mPFC contributes heavily to later DA hypofunction and enhanced psychostimulant responses. Therefore, we are currently testing if pharmacological blockade of mPFC D_2 autoreceptors during adolescent defeat will prevent stress-induced decreases in mPFC DA activity and normalize amphetamine CPP. Establishing this causal link between adolescent social stress, cortical dopamine, and drug seeking is crucial, as it will facilitate development of targeted pharmacotherapies for preventing the onset of drug addiction following teenage bullying.

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Poster

728. Early-Life Stress: Adolescence

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Support: MH57440

Title: Multiple stressors during adolescence induce long-lasting changes in dopamine system responsivity resembling schizophrenia

Authors: *F. V. GOMES, A. A. GRACE;
Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Adolescence is a developmental period of complex neurobiological changes and heightened vulnerability to psychiatric disorders. Evidence suggests that stress during adolescence is an important risk factor in the etiology of schizophrenia, a developmental disorder that typically manifests in late adolescence or early adulthood. Stressful events alter the responsivity of dopamine (DA) system. Additionally, they can precipitate or exacerbate the psychotic symptoms and increase the risk for developing schizophrenia. Thus, we evaluated the

impact of single and combined stressful events during adolescence on schizophrenia-like signs in rats as adults. Male rats were submitted to stressors during adolescence [restraint stress (RS; 1 h session at postnatal day (PD) 31, 32 and 40); footshock (FS; 25 footshocks of 1.0 mA/2s/session daily through PD31-40; or the combination of FS and RS]. At adulthood, animals were tested for anxiety (elevated plus-maze, EPM), cognitive function (novel-object recognition test, NOR) and DA system responsivity [locomotor response to amphetamine and extracellular recordings of ventral tegmental area (VTA) DA neurons]. Electrophysiological parameters evaluated number of DA neurons firing (population activity), their firing rate and amount of burst firing. We also evaluated whether the exposure to the combination of FS and RS in adulthood (PD65-74) produced behavioral and electrophysiological changes similar to the adolescent stressors. All stressors impaired body weight gain and induced anxiety-like responses in the EPM. FS and FS+RS also disrupted cognitive function as assessed by the NOR test. Only animals exposed to the combination of FS and RS showed a DA hyper-responsivity as indicated by augmented locomotor response to amphetamine and an increased number of spontaneously active DA neurons confined exclusively to the lateral VTA, which projects to associative striatal regions analogous to those found to be hyper-responsive in schizophrenia patients. In contrast, no change was observed when rats were exposed to the combination of FS and RS in adulthood, underscoring that adolescence is a developmental period of particular susceptibility. Our results are in agreement with previous studies showing long-lasting changes induced by stressful events during adolescence. The impact on DA system activity, however, seems to depend on higher-level multiple stressors. Hence, stress during adolescence could be a precipitating factor for the transition to schizophrenia later in life, and stress control at this vulnerable period may circumvent these changes to prevent the emergence of psychosis in susceptible individuals.

Disclosures: **F.V. Gomes:** None. **A.A. Grace:** None.

Poster

728. Early-Life Stress: Adolescence

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 728.05/ZZ4

Topic: F.04. Stress and the Brain

Support: NIH Grant RO1 MH73136

NIH Grant RO1 NS28912

NIH Grant P50MS096889

George E. Hewitt Foundation for Biomedical Research postdoctoral fellowship

Title: Adolescent anhedonia provoked by adverse neonatal experience is abrogated by knockdown of amygdala corticotropin-releasing hormone

Authors: *J. L. BOLTON, J. MOLET, L. REGEV, Y. CHEN, D. Z. YANG, T. Z. BARAM; Dept. of Anat. & Neurobio., Univ. of California-Irvine, Irvine, CA

Abstract: Background: Vulnerability to disabling stress-related emotional disorders such as depression is governed in part by early-life experiences. We have previously demonstrated the importance of maternal care, particularly the predictability of sensory signals from the mother, for the maturation of emotional systems within the brain. This long-term programming of risk vs. resilience has been linked to epigenetic reprogramming of specific stress-sensitive neurons, including augmented expression of corticotropin-releasing hormone (CRH) in the central amygdala (ACE). However, the role of augmented CRH in the enduring emotional vulnerability is unclear. We tested if enhanced ACE-CRH following fragmented neonatal experience is necessary for the development of anhedonia, a harbinger of depression.
Methods: We provoked adverse, fragmented neonatal experience by limited bedding and nesting material in the cages (LBN) during (P)2-9, a sensitive developmental period. Adolescents raised in LBN cages had decreased sucrose preference and peer play, indicative of an anhedonia-like phenotype. To determine the role of ACE-CRH in this phenotype, we employed site-specific, siRNA-based knockdown of CRH and determined the effects on emotional outcomes.
Results: The reduction in sucrose preference induced by early-life LBN was completely reversed by ACE CRH knockdown, and analyses of peer play, an independent parameter of pleasure, is ongoing.
Conclusions: Augmented amygdalar CRH is necessary for the development of anhedonia following fragmented neonatal experience. Ongoing studies aim to identify the mechanism of CRH's actions on the neural network underlying reward and anhedonia.

Disclosures: J.L. Bolton: None. **J. Molet:** None. **L. Regev:** None. **Y. Chen:** None. **D.Z. Yang:** None. **T.Z. Baram:** None.

Poster

728. Early-Life Stress: Adolescence

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 728.06/ZZ5

Topic: G.06. Post-traumatic Stress Disorder

Support: Department of Psychology O'Grady Fellowship

Psi Chi Summer Research Grant

CLAS Undergraduate Summer Research and Artistry Fellowship

Title: The impact of environmental enrichment in a rat model of adolescent ptsd

Authors: *E. WALSH[1], A. GARRISON[2], C. UKPABY[2], T. KOELTZOW[2];
[1]Psychology, Bradley Univ., Naperville, IL; [2]Psychology, Bradley Univ., Peoria, IL

Abstract: Post-traumatic stress disorder (PTSD) during adolescence represents an increasingly important diagnostic consideration, as prevalence rates are higher among adolescents compared to adults, and symptom severity and duration may differ significantly (Merikangas et al., 2010). We have recently reported a pre-clinical model of PTSD in rats (Garrison et al., 2015) utilizing a modification of the Single Prolonged Stress (SPS) Model described previously (Liberzon et al., 1997). Results indicate that adolescent rats exhibited robust and prolonged alterations in behavioral anxiety and locomotor activity compared to adults. Environmental enrichment (EE) has been previously shown to decreases anxious behavior and promote exploration of a novel environment (see Simpson & Kelly, 2011). The present study exposed adolescent Sprague-Dawley rats (postnatal day 32-35) to the modified SPS procedure (60 minutes restraint followed by 15 minutes of forced swim) or to sham conditions. Behavioral testing (elevated plus maze, spontaneous locomotor activity, response to novelty) was assessed two weeks after SPS induction. For four weeks, half the subjects were subjected to EE (defined as social housing with novel objects and access to an enriched arena five days/week). All rats were then subjected to another round of behavioral testing. In addition, because substance use disorders are frequently comorbid with PTSD, rats were subsequently tested for the locomotor response to cocaine (15 mg/kg). We have hypothesized that EE will rescue the behavioral consequences of SPS, resulting in increased exploration of the open arms of the elevated plus maze and increased exploration of the open field. In contrast, EE is anticipated to increase the locomotor response to cocaine, presumably due to effects on dopamine transporter expression.

Disclosures: **E. Walsh:** None. **A. Garrison:** None. **C. Ukpaby:** None. **T. Koeltzow:** None.

Poster

728. Early-Life Stress: Adolescence

Location: Halls B-H

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Program#/Poster#: 728.07/ZZ6

Topic: F.04. Stress and the Brain

Support: NIH Grant MH084970

NIH Grant MH109484

Title: Repeated stress impairs prefrontal cortical regulation of the amygdala in rats

Authors: *J. ROSENKRANZ, W. ZHANG;
Cell. and Mol. Pharmacol., The Chicago Med. School/RFUMS, North Chicago, IL

Abstract: The prefrontal cortex (PFC) can impose inhibitory regulation over the activity of the amygdala and thereby regulate emotion. Adolescence is associated with emotional lability and there are hints towards immature PFC regulation over the amygdala in adolescents. Repeated stress produces further dysregulation of emotion. One possible contributing factor for this effect is through impaired control over the amygdala by the PFC. The purpose of this study was to test 1) whether there is less regulation by the PFC over the amygdala in adolescence and 2) whether repeated stress reduces PFC regulation over the amygdala. To accomplish this, adolescent rats were exposed to control handling or repeated restraint stress (P32-41). On P42, rats underwent fear conditioning and extinction or acute in vivo electrophysiological recordings from neurons of lateral (LAT) and basal (BA) nuclei of the amygdala. These adolescent rats were compared to adult rats that were exposed to the same protocol. We found that adolescent rats displayed slower fear extinction, and this was further impaired by repeated stress. In addition, adolescent rats displayed weaker inhibition of LAT and BA neurons upon stimulation of the infralimbic (IL) or prelimbic (PrL) PFC, which was further reduced by repeated stress. Though the mechanism is unclear, these results are consistent with a weaker PFC regulation over the amygdala in adolescent rats, and that this already weaker regulation is disrupted by repeated stress. This may contribute to the harmful effects of repeated stress on affect and mood in adolescence.

Disclosures: J. Rosenkranz: None. **W. Zhang:** None.

Poster

728. Early-Life Stress: Adolescence

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 728.08/ZZ7

Topic: F.04. Stress and the Brain

Support: NIH Grant AA021517

Title: Binge alcohol consumption during puberty causes altered DNA methylation in the brain of alcohol-naïve offspring

Authors: *A. ASIMES, A. R. TORCASO, Y. S. RAO, E. PINCETI, C. K. KIM, T. R. PAK;
Cell and Mol. Physiol., Loyola Univ. Chicago Dept. of Cell and Mol. Physiol., Maywood, IL

Abstract: Binge alcohol consumption among adolescents is a major health concern in the United States, with 21% of teenagers reporting binge-pattern drinking behavior in the last 30 days. Binge drinking is defined as raising the blood alcohol concentration above 0.08% within 2 hours. This behavior is associated with an increased risk for anxiety and mood disorders in adulthood. Children of alcoholics also exhibit a higher rate of suicide as well as an increased risk of alcohol addiction. Recently, our lab has shown that alcohol-naïve offspring of animals exposed to alcohol during adolescence show altered gene expression profiles in the hypothalamus, a region of the brain involved in stress regulation. These intergenerational changes might be caused by a mechanism involving epigenetic inheritance. DNA methylation is a stable and heritable epigenetic mark that can be influenced by environmental factors. Aberrant DNA methylation is implicated in several cognitive disorders that are also associated with alcohol use such as schizophrenia, depression, and addiction. Using a rodent model of adolescent binge alcohol exposure, we employed Enhanced Reduced Representation Bisulfite Sequencing (ERRBS) as an unbiased approach to test the hypothesis that parental exposure to binge-pattern alcohol during adolescence alters DNA methylation in the hypothalamus of alcohol-naïve offspring. Wistar rats were administered a repeated binge-EtOH exposure paradigm where they received 3g/kg of 20% (v/v) EtOH via oral gavage once daily for 3 days, then 2 days vehicle and another 3 days EtOH at both early and late puberty (PND37, 67). Animals were paired for mating 24h after the last EtOH dose. Pairs consisted of all combinations of EtOH- and vehicle-treated males and females. Litters were culled to 10 pups per dam and were left until PND7 when all animals were euthanized. The hypothalamus was extracted from male pups and genomic DNA was isolated. We found that male offspring of alcohol-exposed parents exhibited differential DNA methylation patterns and that these patterns varied between maternal and paternal exposure. Differentially methylated cytosines (DMCs) were distinct between all mating pairs, with only nine genes common to all treatment groups. Genes with DMCs displayed alterations in expression, but methylation was not the only predictor of expression levels, underscoring the complexity of transcriptional regulation in this tissue. Overall, we have shown altered DNA methylation in the hypothalamus of alcohol-naïve offspring following parental adolescent binge drinking.

Disclosures: **A. Asimes:** None. **A.R. Torcaso:** None. **Y.S. Rao:** None. **E. Pinceti:** None. **C.K. Kim:** None. **T.R. Pak:** None.

Poster

728. Early-Life Stress: Adolescence

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Topic: F.04. Stress and the Brain

Support: NIH Grant R01AA021517

Title: Binge alcohol exposure during puberty alters miRNAs and mRNAs involved in synaptic plasticity in the ventral hippocampus

Authors: *Y. S. RAO[1], C. K. KIM[2], A. ASIMES[2], A. TORCASO[2], C. B. DINGWALL[2], T. R. PAK[2];
[1]Cell and Mol. Physiol., Loyola Univ. Chicago Stritch Sch. of Med., Maywood, IL; [2]Cell and Mol. Physiol., Loyola Univ. Chicago, Maywood, IL

Abstract: Puberty is a critical period of postnatal brain development characterized by increased neurogenesis and synaptogenesis. Physical and psychological stressors during pubertal development have been linked with an increased risk for mental health disorders in adulthood, suggesting that perturbations of the hypothalamo-pituitary-adrenal (HPA) axis during this critical period negatively impacts genes regulating these neurodevelopmental processes. Previous studies in our laboratory showed that peri-pubertal episodic binge-pattern alcohol exposure dysregulated HPA axis activity, an effect that persisted in adulthood even after a long period of alcohol abstinence. Further, we showed that alcohol exposure induced long-term alterations in small non-coding microRNAs, some of which were predicted to target key neurodevelopmental genes. The current study extends those findings and we hypothesized that peri-pubertal alcohol exposure dysregulates genes involved in mediating hippocampal synaptogenesis by altering their associated microRNA effectors. To test this hypothesis, peri-pubertal male rats were administered an episodic binge alcohol paradigm as previously described (Przybycien-Szymanska et al., PLoS ONE 2011). Upon euthanasia, the brains were flash frozen and the ventral hippocampus was microdissected. We then used qRT-PCR profiling arrays to measure the expression levels of microRNAs and mRNAs known to be important for neurodevelopment and synaptic plasticity in the ventral hippocampus. Overall, the data revealed that alcohol significantly decreased seven microRNAs (miR-19a/b-3p, miR-29a/c-3p, miR-34a, miR-488-3p, and miR-181d) in the ventral hippocampus. Moreover, alcohol correspondingly increased several genes (mRNAs) associated with synaptic plasticity , suggesting that they were potential targets of the miRNAs that decreased in response to binge alcohol exposure. Together, our data revealed that microRNAs are a putative molecular mechanism mediating alcohol-induced disruption of synaptogenesis during pubertal development.

Disclosures: Y.S. Rao: None. **C.K. Kim:** None. **A. Asimes:** None. **A. Torcaso:** None. **C.B. Dingwall:** None. **T.R. Pak:** None.

Poster

728. Early-Life Stress: Adolescence

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Topic: F.04. Stress and the Brain

Support: NIH Grant R21MH091445

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Klarman Family Foundation Grant Program in Eating Disorders Research

P30 EY13079

Fulbright Graduate Study Grant

Title: Food restriction alone, exercise alone or the two combined during adolescence rescue the behavioral deficits resulting from variant BDNF Val66Met polymorphism

Authors: *Y.-W. CHEN[1], F. LEE[2], C. J. AOKI[1];
[1]Ctr. for Neural Sci., New York Univ., New York, NY; [2]Psychiatry, Weill Cornell Med. Col., New York, NY

Abstract: Brain-derived neurotrophic factor (BDNF) is an important determinant of hippocampal function throughout the lifespan, facilitating neuronal survival and differentiation, synaptic structure and plasticity, long-term potentiation, learning and memory. A single nucleotide polymorphism (SNP), which produces a valine-to-methionine substitution at codon 66 (Val66Met) in BDNF leading to reduced activity-dependent secretion of BDNF. The BDNF Val66Met polymorphism has been associated with anxiety disorder, and abnormalities in hippocampal formation and cognitive function. Previous studies suggest that both food restriction and exercise elevate BDNF levels in the hippocampus. Here, we investigated whether food restriction and/or exercise during adolescence would rescue the behavioral deficits in BDNF-Val66Met knock-in mice. Four experimental groups of male and female pubertal BDNF-Val66Met knock-in mice and their wild-type littermates were evaluated: CON (singly housed mice with no running wheel experience or food restriction), and three experimental groups: EX (housed with a wheel from P36-44), FR (food restricted from P41-44), and FR-plus-EX (housed with a running wheel from P36-44 and food restriction from P41-44). We assessed anxiety-related levels of these animals after recovery from food restriction by elevated plus maze. Spatial and object recognition memory and social preference and recognition of the animals were also tested after recovery from food restriction. Our preliminary data suggest that, during early adulthood, male $BDNF^{Met/Met}$ mice display increased anxiety-like level and show impaired spatial, object recognition memory, and social recognition ability. EX, FR, and FR-plus-EX

during adolescence have no effect on anxiety-like level in $BDNF^{Met/Met}$ mice. However, EX, FR, and FR-plus-EX show differential effects but mostly rescue the spatial, object recognition memory, and social recognition deficits of $BDNF^{Met/Met}$ mice to levels of $BDNF^{Val/Val}$ controls. On the other hand, female $BDNF^{Met/Met}$ mice display normal spatial memory and social recognition ability, but increased anxiety-like level and impaired object recognition memory. EX, FR, and FR-plus-EX can rescue the object recognition memory deficits in $BDNF^{Met/Met}$ female mice. This study highlights sex differences in the effects of BDNF Val66Met polymorphism on cognitive functions, while anxiety-like behavior is similar across the sexes. This study also provides evidence for the effects of food restriction and/or exercise during adolescence on rescuing the behavioral deficits in $BDNF^{Met/Met}$ mice.

Disclosures: Y. Chen: None. **F. Lee:** None. **C.J. Aoki:** None.

Poster

728. Early-Life Stress: Adolescence

Location: Halls B-H

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Program#/Poster#: 728.11/ZZ10

Topic: F.04. Stress and the Brain

Support: IMHR Graduate Student Research Bursary

Title: Juvenile stressor exposure and diet alters ghrelin sensitivity in adulthood

Authors: *E. ALI[1,2], P. KENT[2,3], A. FREITAS[1], A. AWADIA[3], M. ABU NADA[3], J. JAMES[2], A. ABIZAID[1], Z. MERALI[2,3];
[1]Carleton Univ., Nepean, ON, Canada; [2]Inst. of Mental Hlth. Res., Ottawa, ON, Canada; [3]Univ. of Ottawa, Ottawa, ON, Canada

Abstract: Juvenile stress is proposed to contribute to the prevalence of obesity in school-aged children by possibly increasing the preference and consumption of calorically dense palatable food (PF). This stressed-induced consumption of PF may lead to long-term detrimental consequences such as obesity in adulthood. There is evidence to suggest that ghrelin, an orexigenic peptide, contributes to this stress-induced preference by mediating dopamine release within mesolimbic structures. More specifically, ghrelin binds to the growth hormone secretagogue receptor which are expressed at the ventral tegmental area and can stimulate dopamine release to the nucleus accumbens. Ghrelin increases reward-associated behaviors such as conditioned place preference for drugs of abuse, motivation and enhancing cocaine hyperlocomotor activity. As such, the objective of the present study was to assess how access to PF in combination with juvenile stressor exposure alters sensitivity to exogenous administered

ghrelin. In this study, two groups of male Wistar rats were subjected to an episodic stressor exposure during the juvenile period on post-natal days (PD) 27-29. Both groups received ad lib access to chow however one group had daily limited access to PF. In addition, there were two non-stressed diet counterpart groups. In adulthood (PD 61), rats were subjected to a central injection (ghrelin (10ug) or vehicle) and given ad lib access for two hours to one of the two diets: a novel palatable diet (cookie dough) or chow. Consumption was recorded. Afterwards, locomotor activity was compared between an intraperitoneal injection of cocaine (10mg/kg) with and without a peripheral pretreatment of ghrelin (10ug). Our findings indicate that in adulthood, central ghrelin administration did not increase consumption of cookie dough in PF fed rats regardless of stressor exposure, however in previously stressed chow fed rats an increase in cookie dough intake was observed. Interestingly, a pretreatment of ghrelin did not increase cocaine hyperlocomotor activity in PF fed rats; yet enhanced cocaine hyperlocomotor activity was displayed in chow fed rats. These findings suggests that access to PF dampens ghrelin signaling regardless of juvenile stressor exposure and that juvenile stressor exposure enhances ghrelin signaling in chow fed rats. It is proposed that stressor exposure during this sensitive developmental period can elicit a sensitization to rewarding stimuli (PF and drugs of abuse) in adulthood that is mediated by enhanced ghrelin signaling.

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Poster

728. Early-Life Stress: Adolescence

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Topic: F.04. Stress and the Brain

Support: NSERC Discovery Grant, RGPIN 249685

Title: Effects of juvenile stress exposure on adult behaviour and physiology in convict cichlid fish

Authors: *B. HOPE[1], K. FJELLNER[2], N. BATTY[1], P. HURD[2];
[2]Psychology, [1]Univ. of Alberta, Edmonton, AB, Canada

Abstract: Stress coping style is a major personality dimension in fish and may be assessed using behaviours related to anxiety. Anxiety is indicated by freezing and hiding behaviour, while ambulatory behaviour indicates a lack of anxiety. Moderate stress exposure during development can result in stress inoculation, such that individuals display more active coping styles compared

to those without stress exposure. This study investigates how developmental stress exposure affects adult stress coping styles in the convict cichlid (*Amatitlania nigrofasciata*) to explore possible stress inoculation effects on behavioural and physiological responses to stress. Fish were net chased for two minutes per day for two weeks immediately after becoming free-swimming, and then their behavioural (i.e., exploration patterns in open field and plus maze tasks) and cortisol responses to stress were assessed at nine months of age. Cortisol levels by weight were significantly correlated to treatment, such that stressed fish had lower cortisol levels than controls. This result is consistent with the stress inoculation hypothesis that moderate juvenile stress exposure will result in lower stress reactivity (i.e., lower cortisol levels) later in life. In the plus maze task, there was a significant effect of treatment on the amount of time spent in open and closed arms but no effect of cortisol levels. Stressed fish spend more time in open arms than control fish, which is also consistent with stress inoculation, as the stressed fish are employing a more active coping style than the control fish. In contrast to plus maze, open field task showed a significant relationship between amount of time spent in inside squares and cortisol levels and no effect of treatment. Fish with lower cortisol levels spent more time in inside squares, while fish with higher cortisol levels spent less time in inside squares. A significant interaction effect between cortisol levels and treatment on exploration rate of inside squares, such that stressed fish with low cortisol levels explored faster and those with high cortisol levels explored slower, while control fish had medium exploration rates independent of cortisol levels. It is possible that stress exposure influences the hormonal profile, as expected by the relationship between cortisol levels and treatment, which is supported by the interaction effect on exploration rate. This suggests that plus maze behaviour may be affected more by personality, such as anxiety, while open field may be more influenced by physiology, such as hormonal profiles. Further research will be done to delve deeper into this dichotomy.

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Poster

728. Early-Life Stress: Adolescence

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Title: Food restriction-evoked voluntary exercise increases the number of varicosities and average lengths of noradrenergic axons in the hippocampal hilus of adolescent female rats

Authors: *A. EVANS, A. AKAD, Y.-W. CHEN, C. AOKI;
Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: Anorexia nervosa (AN) is a disorder characterized by intentional food restriction, severe weight loss and excessive exercise and is often comorbid with anxiety and depression. The onset of AN is most often during adolescence, a period of heightened neurogenesis in the dentate gyrus. Little is yet known about the structural and developmental impact of AN on adolescent brains. While it has been shown that the peripheral noradrenergic (NA) system is perturbed in patients diagnosed with AN, whether the NA system within brain is also altered has not been reported. In order to explore brain changes that could be associated with AN, we used an animal model, called activity-based anorexia (ABA), whereby food restriction evokes voluntary food restriction and excessive exercise (possibly, as a form of foraging behavior) within 24 hrs, causing some to die unless removed from the food restriction-plus-wheel environment. Animals' daily wheel running distances and body weights were measured for 8 days, from P35 or 36. Food restriction was imposed during the last 4 of those 8 days. The extent of weight loss and increase in wheel running during the food-restricted period varied across individuals, with weight loss ranging from 11% to 23% and wheel activity increasing 0 to > 6-fold among 8 ABA rats. On P44, all animals were euthanized by transcardial perfusion with 4% paraforlamdehyde. Brain tissue was immunolabeled for norepinephrine's rate-limiting synthetic enzyme, dopamine β-hydroxylase. Neurolucida 360's AutoNeuron feature was used to automatically trace, then count the number and measure lengths of NA axons contained within the hilus from coronal vibratome sections (10 µm x 131.5 µm x 175.5 µm). Neurolucida (version 10) was also used to manually trace 10 randomly selected axons per animal, together with all of their associated varicosities, within the hilus. Compared to age-matched rats without food restriction or wheel access (N=8, CON), the inter-varicosity distances along NA axons was reduced by 26% ($p<0.05$) within the hilus of ABA animals, while the average axonal length was increased by 34% ($p<0.05$), together increasing the potential release sites of norepinephrine. Pearson correlation analyses revealed that resilient animals, characterized by minimal weight loss or minimal FR-evoked running, exhibit more varicosities ($r=.67$, $p=.07$) and longer axonal segments ($r=-.69$, $p=0.05$). Thus, individuals with the greatest adaptability for survival by suppressing wheel running are also the ones able to increase NA innervation the most. Enhanced NA signaling may support behavior that is adaptive to food deprivation within confine of a cage, namely to eat, rather than run.

Disclosures: **A. Evans:** None. **A. Akad:** None. **Y. Chen:** None. **C. Aoki:** None.

Poster

728. Early-Life Stress: Adolescence

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Topic: F.04. Stress and the Brain

Support: Grinnell College

Title: Acute corticosterone treatment increases anxiety and dendritic elongation and arborization in the orbitofrontal cortex in mid-adolescent but not early-adolescent male rats

Authors: *N. L. REMPEL-CLOWER, T. EARNEST, M. YETTER;
Grinnell Col., Grinnell, IA

Abstract: Stress during adolescence is a risk factor for anxiety and mood disorders, and has been shown to induce morphological changes in neurons in the prefrontal cortex and amygdala. As in adults, adolescent exposure to stressors activates the HPA axis, leading to increased corticosterone (CORT) levels in rats, and the hormonal response to stress is enhanced during adolescence. Previous research has found varying effects of exposure to stressors on anxiety associated with age of exposure and age of testing. Although the orbitofrontal cortex (OFC) and basolateral amygdala (BLA) participate in managing cognitive and emotional responses to stress, little is known about how acute stress during specific periods of adolescence affects the dendritic structure of neurons in these regions. The aim of the present study was to elucidate age-dependent effects of acute CORT treatment during adolescence. Specifically, we investigated if a single CORT injection increases anxiety-type behavior in adolescent rats and if it causes OFC and BLA dendritic remodeling. Male adolescent Sprague-Dawley rats were given CORT (10 mg/kg) or saline control injections in early adolescence (28 days old) or given CORT or saline control injections in mid-adolescence (36 days old). Anxiety was assessed with the elevated plus maze and open field test 12 days after injection, while rats in both groups were still within the adolescent period. One day after behavioral testing, rats were euthanized and brain tissue was Golgi-stained for assessment of dendritic morphology of neurons in the OFC and BLA using Neurolucida. In the elevated plus maze, mid-adolescent CORT-treated rats spent significantly more time in the open arms than the age-matched control group, indicating an increase in anxiety-type behavior. Early-adolescent CORT-treated rats, in contrast, did not exhibit any behavioral effects of the treatment. Similar age-specific effects of CORT were observed in OFC neuronal structure. In the mid-adolescent CORT-treated rats, morphological analysis revealed evidence of elongation and arborization of apical dendrites of OFC neurons. No changes were detected in basal dendrites of these neurons. CORT treatment during early adolescence had no effect on dendritic morphology of OFC neurons. In BLA neurons, no changes in dendritic morphology were observed after CORT treatment during either early adolescence or mid-

adolescence. Taken together, these findings suggest that acute CORT administration in mid-adolescence causes increased anxiety and dendritic expansion in the OFC later in adolescence, but these effects are not present after acute CORT administration in early adolescence.

Disclosures: N.L. Rempel-Clower: None. **T. Earnest:** None. **M. Yetter:** None.

Poster

728. Early-Life Stress: Adolescence

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Topic: F.04. Stress and the Brain

Support: MH093981 to Rita Valentino

NIH 5T32NS007413-17 to Kim Urban

Title: Divergent morphological effects of repeated social stress on rat prefrontal cortical pyramidal neurons in adolescent compared to adult male rats.

Authors: *E. GENG;
Children's Hosp. of Philadelphia, Philadelphia, PA

Abstract: Chronic stress can lead to psychiatric illness characterized by impairments of executive function, implicating the prefrontal cortex as a target of stress-related pathology. Previous studies have shown that multiple types of chronic stress reduce dendritic branching, length, and spines of medial prefrontal cortex (mPFC) pyramidal neurons. However, these studies focused on layer 2/3 pyramidal neurons in adult rats. Whereas these neurons process incoming information, layer 5 is the major output layer. Because the prefrontal cortex develops throughout adolescence, stress during this period may have a greater impact on structure and function than stress during adulthood. In this study, male adolescent (42-48 days old; n = 36 neurons from 4 controls, 30 neurons from 4 stressed rats) or adult (68-72 days old; n=37 neurons from 4 controls, 32 neurons from 4 stressed) rats were exposed to repeated social stress as 5 days of resident-intruder stress or control manipulation. Brains were processed for Golgi staining 24 h after the final manipulation, cells were visualized on a Nikon Eclipse scope, and Neurolucida was used to reconstruct and analyze dendrites. Repeated social stress induced age- and layer-specific effects. In layer 2/3, there was an interaction of age*stress such that stress increased basal dendritic length in mid-adolescents, but decreased it in adults [$F(3,119)=5.38$, $p=0.0003$]. There was an overall stress-induced decrease in basal dendrite branching [stress effect: $F(3,119)=6.15$, $p<0.0001$]. Mid-adolescent neurons had significantly more apical branches than

adults [age effect: F(3,119)=11.85, p=0.003] and stress decreased apical branches in mid-adolescents but not adults [age*sex interaction: F(3,119)=11.85, p=0.014). In layer 5, basal dendrites of mid-adolescents were longer [age effect: F(3,134)=16.44, p<0.0001 and had more branches [age effect: F(3,134)=17.56] Stress increased basal dendrite length [age*stress interaction: F(3,134)=16.44, p=0.0004] and branching [age*stress interaction: F(3,134)=17.56, p<0.0001] only in adults, and decreased branching in mid-adolescents. Stress also increased apical dendrite length only in adults [age*stress: F(3,134)=6.35, p=0.0028] . Likewise, stress increased apical branching in adults but decreased it in mid-adolescents [F(3,134)=7.08, p=0.0002]. These findings demonstrate divergent morphological effects of social stress on mPFC dendritic structure of adolescent and adult male rats that suggest an increased vulnerability of adolescents to cognitive-impairing effects of social stress and the development of an opposing response that may be adaptive in adulthood.

Disclosures: E. Geng: None.

Poster

728. Early-Life Stress: Adolescence

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 728.16/AAA1

Topic: F.04. Stress and the Brain

Support: NSF GRFP Grant No. (DGE-1444932)

NIH MH101477

Title: TrkB stimulation blocks long-term behavioral consequences of adolescent corticosteroid exposure

Authors: *E. T. BARFIELD[1], K. J. GERBER[2], S. L. GOURLEY[3];
[1]Grad. Program in Neuroscience, Yerkes NPRC, [2]Mol. & Systems Pharmacol., [3]Grad. Program in Neuroscience, Yerkes, Dept of Pediatrics, Dept of Psych & Behavioral Sci., Emory Univ., Atlanta, GA

Abstract: In humans and rodents, stress regulates reward-related decision-making. For instance, prolonged stressor and corticosteroid exposure impair the ability of rodents to select actions based on their consequences. However, neurodevelopmental factors remain largely uncharacterized. Here, we exposed mice to oral corticosterone (CORT) and found that subchronic exposure in early adolescence, but not in adulthood, impaired goal-directed decision-making in adulthood. The pattern of behavioral deficiencies was reminiscent of that following

hippocampal damage, so we characterized the expression of tyrosine receptor kinase B (trkB) in the ventral hippocampus (vHC) and interconnected regions – the medial prefrontal cortex and amygdala – following CORT. CORT exposure decreased the ratio of the full-length (active) trkB receptor to the truncated (inactive) form of trkB in all regions and in both adolescents and adults. However, CORT decreased phosphorylation of the signaling protein ERK1/2 (downstream of trkB) selectively in the vHC of adolescent mice. Administration of the trkB agonist, 7,8-dihydroxyflavone (7,8-DHF), in adolescence blocked CORT-induced decision-making and motivational deficits in adulthood in both males and females and induced resilience to depressive-like behavior in the forced swim test. These findings implicate vHC trkB-ERK1/2 signaling in habit-based behaviors and motivational deficiencies following stress hormone exposure in early adolescence.

Disclosures: **E.T. Barfield:** None. **K.J. Gerber:** None. **S.L. Gourley:** None.

Poster

728. Early-Life Stress: Adolescence

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 728.17/AAA2

Topic: F.04. Stress and the Brain

Support: Predoctoral Fellowship 646 from COLCIENCIAS

Title: Stress in early puberty produces impulsive quitters

Authors: *L. F. GONZALEZ MARTINEZ, J. D'AIGLE, S. LEE, H. J. LEE, Y. DELVILLE; Psychology, The Univ. of Texas At Austin, Austin, TX

Abstract: Traumatic experiences during childhood or adolescence can alter behavioral responses and are a risk factor for mental disorders later in life. In animals, individuals attacked by adults during puberty become themselves aggressive once adults. Perhaps, enhanced aggression observed as repeated attacks towards opponents is associated with a lack of impulse control or a deficit in adjusting behavior when environmental rules change (behavioral flexibility). We analyzed impulsivity and behavioral flexibility in male golden hamsters exposed daily to aggressive adults from postnatal day 28 to 42. Later in adulthood, the animals were tested in a Go-NoGo task to evaluate action inhibition. In the Go-NoGo task subjects were trained to respond to a “go” cue (lever press in the presence of a light) and inhibit response to the “no-go” cue (withhold lever press in the presence of a combined tone and light cue). Previously stressed hamsters were less likely to inhibit a conditioned lever pressing response during NoGo trials. Since this effect could be the result of a lack of behavioral flexibility, the animals were tested to

evaluate their response when the reward associated with conditioned lever presses was fully omitted. However, all animals were equally capable of changing their behavior and decrease the frequency of conditioned response across sessions. Behavioral flexibility was further addressed by testing responses when a previously immediate reward was delayed by 60 seconds after lever pressing. Previously stressed animals showed decreased lever pressing on the first day of testing, abandoning the chamber area with the lever within minutes. These studies show that stress in early puberty causes a reduction in action inhibition as seen in the Go-NoGo task and selective impairment in behavioral flexibility as seen when the reward was delayed but not when it was omitted.

Disclosures: L.F. Gonzalez Martinez: None. **J. D'Aigle:** None. **S. Lee:** None. **H.J. Lee:** None. **Y. Delville:** None.

Poster

729. Neurovascular and Metabolic Coupling: Normal Brain and Stroke

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 729.01/AAA3

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: Heart and Stroke Foundation of Canada

Fonds de la recherche en santé du Québec

Canadian Foundation for Innovation

Canadian Institutes of Health Research

Title: Impact of arterial stiffness on cognitive and cerebrovascular functions

Authors: *M. F. IULITA[1], G. MUHIRE[1], D. VALLERAND[1], M. GRATUZE[2], F. R. PETRY[2], G. FERLAND[1], E. PLANEL[2], H. GIROUARD[1];
[1]Univ. De Montreal, Montreal, QC, Canada; [2]Univ. Laval, Quebec, QC, Canada

Abstract: Arterial stiffness is a common condition associated to aging and hypertension, and it is emerging as a strong risk factor for cognitive decline and dementia. Arterial stiffness refers to the reduced capability of an artery to buffer the pulsatile blood flow from ventricular ejection. When large arteries loose elasticity, the resultant increased pulsatility may lead to downstream microvascular damage. The cellular and molecular mechanisms linking arterial stiffness to cognitive dysfunction remain poorly understood. Thus, using a novel murine model based on carotid calcification, we aimed to examine the effects of arterial stiffness on learning and

memory, neurovascular coupling, blood-brain barrier (BBB) permeability and features of Alzheimer's disease pathology. Arterial stiffness was induced by the application of a 0.3M $CaCl_2$ –soaked pad on the right carotid artery (20 min) of C57BL/6 male mice aged 10-12 weeks. Control mice received 0.9% NaCl, in identical conditions. Animals were sacrificed between 2 and 3 weeks after surgery. Results show that carotid calcification leads to a slower spatial learning acquisition, as assessed by the Morris water maze test. In this paradigm, mice with arterial stiffness further exhibited significant impairments in spatial reference memory. Arterial stiffness also attenuated the cerebral blood flow response to whisker stimulation and to the topical application of the endothelium-dependent vasodilator acetylcholine, monitored by laser-Doppler flowmetry *in vivo*. Although modest, arterial stiffness led to a shift in the Aβ40/Aβ42 ratio in the frontal cortex, without affecting tau protein phosphorylation. Analysis of cerebral autoregulation and BBB permeability are underway. These initial results show that arterial stiffness has a negative impact on cognitive and cerebrovascular functions, and should therefore be considered as target to protect the brain. In the long-term, we plan to use this model to test experimental therapeutics.

Disclosures: **M.F. Iulita:** None. **G. Muhire:** None. **D. Vallerand:** None. **M. Gratuze:** None. **F.R. Petry:** None. **G. Ferland:** None. **E. Planel:** None. **H. Girouard:** None.

Poster

729. Neurovascular and Metabolic Coupling: Normal Brain and Stroke

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 729.02/AAA4

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: NSF # HRD-1363399 and HRD-1463889.

Title: Is cerebral hemodynamic variability needed to maintain cognition?

Authors: *S. KESLACY, G. ACOSTA, J. RAMIREZ, J. BENAVIDEZ, C. DY; Kinesiology, CSULA, Los Angeles, CA

Abstract: Acute exercise has been recognized to modulate cerebral blood flow (CBF), but the relationship between changes in CBF and cognitive performance is unclear. **Purpose:** (i) to assess the effects of exercise intensity on CBF (ii) to assess the effects of exercise on cognitive performance (iii) to determine the relationship between exercise-induced changes in CBF and cognition. **Methods:** 31 sedentary adults (19 males and 12 females, 26 ± 3 yrs.) participated in a crossover study and performed a light (20% of power max) and moderate (65% of power max) constant-load cycling exercise for 10 minutes. Middle cerebral artery (MCA) blood flow velocity

(CBFv), peak and pulsatility index (PI) were continuously monitored during exercise using transcranial Doppler ultrasonography. Cognition was assessed using the Cogstate brief battery test: detection task (for psychomotor function / speed of processing), identification task (for visual attention), one card learning (for visual learning & memory), one back task (for working memory) and Groton maze (for visuospatial memory) were performed before and immediately after exercise. **Results:** CBFv increased significantly during exercise at 20% ($p=.02$) and tend to decrease during exercise at 65% ($p=.06$). CBFv significantly increased during the Groton Maze test at rest ($p=.02$) but not during any other cognitive test. There were no changes in CBFv during the cognitive testing following exercise at 20%, however, CBFv decreased and PI increased ($p<0.01$ and $p<0.04$ respectively) for all the cognitive testing following the exercise at 65%. Cognitive performance did not change following exercise despite the change in CBFv. **Conclusion:** Exercise regardless of intensity did not result in changes in cognitive performance. Thus, CBF hemodynamic variability may be necessary to preserve cognitive performance.

Disclosures: S. Keslacy: None. **G. Acosta:** None. **J. Ramirez:** None. **J. Benavidez:** None. **C. Dy:** None.

Poster

729. Neurovascular and Metabolic Coupling: Normal Brain and Stroke

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 729.03/AAA5

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: National Science Foundation Grant # HRD-1302873

CSU Office of the Chancellor.

Title: Sex and intensity modulate cerebral hemodynamic symmetry during exercise

Authors: *J. RAMIREZ, J. BENAVIDEZ, G. ACOSTA, C. DY, S. KESLACY;
Kinesiology, California State Univ. Los Angeles, Los Angeles, CA

Abstract: A very tight cerebral blood flow (CBF) hemodynamic response is essential for brain function. Most of the studies on CBF using the middle cerebral artery (MCA) have been investigating only one hemisphere (predominantly right). Thus, lateralization in blood flow response to stimulus is not well understood. At rest, CBF symmetry is expected; whereas asymmetrical flow can be an indicator of psychological or physiological pathology. It is possible that asymmetries in cerebral hemodynamics also exist as a normal and healthy response to stimulation in order to maintain cognition. **Purpose**: To assess bilateral symmetry in MCA blood

flow during a physiological stress. **Methods**: Participants (N = 33, 15 femals and 18 male) performed 10 minutes of light (20% of max power) and moderate (65% of max power)cycling exercise. Brain hemodynamics measures of mean velocity, peak velocity, and pulsatility index were monitored bilaterally for the MCA by two 2-MHz Doppler probes. Sample sizes were set to 10 mm and depth ranged from 50 to 60 mm. Data was processed using commercially available software (Sonara Medical Systems, Carefusion). Statistical significance in bilateral asymmetry was assessed using a mixed model analysis of variance with gender (male vs female) and exercise intensity (light vs moderate) as between-subjects factors (IBM, SPSS) and significance was set as $p < 0.05$. **Results**: At rest, no significant differences in right versus left CBF mean velocity (mean = 50.5 vs. 46.4 cm s^{-1}), peak (mean = 80.3 vs. 78.2 cm s^{-1}), or pulsatility index (mean = 1.1 vs 1.2) were observed ($p > 0.05$ for all measures). During exercise, assessment for the main effect of exercise intensity revealed significantly greater right compared to left side peak velocity during moderate exercise ($p < 0.05$), but not during light exercise. No significant gender-specific asymmetries were detected as main effects. A significant interaction amongst right/left asymmetry, exercise, and gender was detected in both mean velocity and peak velocity ($p < 0.05$). Post-hoc analysis revealed greater right compared to left CBF mean velocity and peak velocity during light exercise in females ($p < 0.05$ for both measures). The same was also observed in males, but for moderate intensity exercise ($p < 0.05$ for both measures). **Conclusion**: Hemispheric differences in MCA blood flow exists in response to metabolic stress, although level of intensity and gender can influence the nature of the asymmetry in cerebral hemodynamics. These findings lead the way for future study to better understand mechanisms driving cerebral blood flow asymmetry during exercise.

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Poster

729. Neurovascular and Metabolic Coupling: Normal Brain and Stroke

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 729.04/AAA6

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Title: Regional CNS differences in rapid oxygen and pH changes during neurovascular coupling determined using fast-cyclic voltammetry

Authors: *P. S. HOSFORD[1], J. MILLAR[2], J. WELLS[1], I. N. CHRISTIE[1], A. V. GOURINE[1]; [1]Univ. College, London, London, United Kingdom; [2]Barts and The London Sch. of Med. and Dent., London, United Kingdom

Abstract: The mechanisms of neurovascular coupling (NVC) match brain energy supply with demand. The molecular events that trigger functional hyperemia are under debate, and may not be regionally uniform. There is a lack of experimental techniques with sufficient spatio-temporal resolution to probe changes in parenchymal O_2 concentration, PCO_2 and pH at the level of individual CNS nuclei. Here we used fast-cyclic voltammetry (FVC) to monitor changes in tissue PO_2 and pH in the somatosensory cortex and brainstem during neuronal activation *in vivo*. A voltage ramp is applied to carbon fiber microelectrodes (CFM; ⌀ 7μm) from 0v to -1v at 200V/s, 4 times every second. Molecular oxygen and protonated groups on the surface of the CFM are reduced. Currents generated from these reactions are proportional to the concentration of oxygen and protons. Adult male rats (~300g) were anesthetized with α-chloralose (75mg kg^{-1}). Blood gas tensions and pH were maintained within the physiological ranges. CFM was placed in the somatosensory region of the cortex or in the brainstem's nucleus of the solitary tract (NTS). Electrode placements were confirmed by recording extracellular potentials evoked by the electrical stimulation of the forepaw or the vagus nerve (1mA, 1Hz). Exposed tissue was sealed from the air.

To confirm voltammetric oxygen and pH sensitivities, systemic hypoxia and hypercapnia were induced. During apnea (30s) expected rapid decreases in cortical brain tissue PO_2 were recorded(n=3). Systemic hypercapnia (10% CO_2; 5min) caused a decrease of brain parenchymal pH by 0.17±0.04 units (n=5).

Electrical forepaw stimulation evoked biphasic changes in cortical parenchymal pH and PO_2. Initially, PO_2 increased (by 12±1mmHg; n=6), followed by a decrease of 8±3 mmHg. This profile of PO_2 changes was similar to the time course of BOLD signal recorded under similar experimental conditions in a 9.4T small animal MRI scanner (Agilent). Changes in pH followed a similar profile with an initial increase (by 0.1±0.02 units; n=6) followed by a decrease (by 0.06±0.01 units) before returning to the baseline. In the brainstem, electrical stimulation of the vagus nerve triggered an initial decrease in PO_2 (by 7±2mmHg; n=5) followed by an increase (by 3±1 mmHg; n=5). pH decreased (by 0.15±0.04 units) before returning to the baseline.

These data demonstrate that pH and PO_2 changes associated with NVC vary between different brain regions. FCV allows simultaneous electrochemical detection of blood flow-related changes in brain parenchymal PO_2 and pH with sufficient spatio-temporal resolution required to investigate the physiological significance of these differences.

Disclosures: P.S. Hosford: None. **J. Millar:** None. **J. Wells:** None. **I.N. Christie:** None. **A.V. Gourine:** None.

Poster

729. Neurovascular and Metabolic Coupling: Normal Brain and Stroke

Location: Halls B-H

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Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH Grant R01NS078168

NIH Grant R01NS079737

McKnight Endowment: Scholar Award

American Heart Association: National Development Grant

Title: Coupling of spontaneous and sensory evoked hemodynamic signals to neural activity in the barrel cortex of awake mice

Authors: *A. WINDER[1], P. J. DREW[2,3,4];
[2]Engin. Sci. and Mechanics, [3]Dept. of Neurosurg., [4]Dept. of Bioengineering, [1]Pennsylvania State Univ., University Park, PA

Abstract: Hemodynamic signals are used to infer neural activity in the absence of overt behavior or tasks using non-invasive imaging techniques such as fMRI. However, it is not clear whether spontaneous neural activity and sensory evoked neural activity are coupled to hemodynamic changes in the same way. We simultaneously measured neural activity, cerebral blood volume (CBV), and behavior in the somatosensory cortex of awake, head-fixed mice in order to compare neurovascular coupling across behavioral states. Whisker position was monitored to detect periods of attentive active sensing and the whiskers were periodically stimulated with brief puffs of air. This allowed the measured CBV and neural data to be categorized according to three behavioral types: sensory-evoked, volitional whisking, and rest, which was defined as the absence of discernible behaviors. The variance in CBV was significantly larger during passive sensation and volitional movement than during rest. The hemodynamic response function (HRF), which is the linear kernel that relates neural activity to the measured CBV, was similar for all three behaviors. This indicates that neurovascular coupling is conserved across behaviors, but that large "spontaneous" CBV changes are driven by volitional movement. To determine the amount of CBV variance explained by neural activity, the HRF was used to predict CBV changes from the measured local neural activity on individual trials and for averaged data. The HRF performed well at predicting sensory evoked CBV changes, accounting for ~80% of the CBV variance. However, <10% of the CBV fluctuations at rest could be accounted for by the local neural activity. The unpredicted component of the CBV fluctuations was concentrated around 0.1 Hz. To test whether a portion of resting-state CBV was driven independently of local

neural activity, we infused muscimol into the barrel cortex while measuring CBV and neural activity. Muscimol decreased the amplitude of sensory-evoked CBV response and the CBV variance at rest, confirming that spontaneous hemodynamic fluctuations depended strongly on local neural activity. The power of remaining CBV fluctuations was concentrated at 0.1 Hz. Our results suggest that large spontaneous hemodynamic signals are driven by volitional, active behaviors, and confirm that spontaneous hemodynamics are associated with local neural activity. However, the poor performance of the HRF during rest suggests a complex relationship between measured neural activity and spontaneous hemodynamic fluctuations.

Disclosures: **A. Winder:** None. **P.J. Drew:** None.

Poster

729. Neurovascular and Metabolic Coupling: Normal Brain and Stroke

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Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

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Title: Pericyte degeneration leads to neurovascular uncoupling

Authors: *K. KISLER[1], A. R. NELSON[1], S. V. REGE[1], A. RAMANATHAN[1], Y. WANG[1], A. AHUJA[1], D. LAZIC[1,2], P. S. TSAI[3], Z. ZHAO[1], Y. ZHOU[4], D. A. BOAS[5], S. SAKADŽIĆ[5], B. V. ZLOKOVIC[1];
[1]Dept. of Physiol. and Biophysics, Zilkha Neurogenetic Inst., Keck Sch. of Med. of the Univ. of Southern California, Los Angeles, CA; [2]Dept. of Neurobiology, Inst. for Biol. Res., Univ. of Belgrade, Belgrade, Serbia; [3]Dept. of Physics, UCSD, La Jolla, CA; [4]Dept. of Neurobiology, Chongqing Key Lab. of Neurobio., Third Military Med. Univ., Chongqing, China; [5]Optics

Division, Athinoula A. Martinos Ctr. for Biomed. Imaging, Dept. of Radiology, Massachusetts Gen. Hosp. and Harvard Med. Sch., Charlestown, MA

Abstract: The regulation of cerebral blood flow (CBF) and oxygen supply to match neuronal functional activity is regulated by synchronous action of different cell types -- neurons, vascular cells such as vascular smooth muscle cells and endothelium, and glia -- comprising the neurovascular unit. Pericytes, mural cells of the capillary vessel wall, are critical for the stabilization of the capillary wall, maintenance of the blood-brain barrier and have recently been implicated in the regulation of capillary diameter. However, their role in regulation of neurovascular coupling remains debatable. Using pericyte-deficient Pdgfrβ+/- mice, we show that pericyte reduction *in vivo* leads to delayed capillary dilation in response to neuronal stimulus, and reduced red blood cell flow velocity in capillaries carrying oxygen to activated brain sites, in spite of intact arteriolar response and vasoactivity. We show that diminished cortical CBF responses to stimulus in pericyte-deficient mice leads to metabolic stress, which over time leads to impaired neuronal excitability and neurodegenerative changes. Together, these data imply that pericytes play an important role in neurovascular coupling and oxygen supply to the brain, and that pericyte degeneration contributes to neurovascular uncoupling. Degeneration of pericytes, likely contributes to neurovascular dysregulation, leading to neurovascular dysfunction and eventually neurodegeneration.
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Poster

729. Neurovascular and Metabolic Coupling: Normal Brain and Stroke

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

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Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: Royal Society University Research Fellowship (CM)

Wellcome Trust Research Project Grant (CM/AS)

Wellcome Trust Sir Henry Wellcome Fellowship (CH)

BBSRC Research Grant (JB/CM)

Title: Serotonergic modulation of neurovascular coupling: receptor and pathway specific effects.

Authors: A. SPAIN, G. BREZZO, J. BERWICK, C. HOWARTH, *C. J. MARTIN;
The Univ. of Sheffield, Sheffield, United Kingdom

Abstract: The rapid and local adjustment of cerebral blood flow in relation to changing neuronal activity is termed neurovascular coupling. Whilst our understanding of the physiological mechanisms underpinning neurovascular coupling has advanced substantially in recent years, empirical work has focussed on the role for excitatory, glutamate-mediated neurotransmission in neurovascular coupling, with little research into how important modulatory neurotransmitters such as serotonin (5-HT) might affect neurovascular function. Receptors for 5-HT are found not only upon many neuronal cell types, but also upon non-neuronal neurovascular unit cells. Changes in brain 5-HT and/or in the availability of specific 5-HT receptors has the potential to alter neurovascular function in complex ways which might be dependent upon brain region, neurophysiological (activation) conditions, and neuropathology. To investigate this, we used a rodent model in which acute pharmacological manipulation of the 5-HT system was combined with multi-modal neuroimaging and electrophysiological recording. This enabled us to determine neurovascular coupling relationships in-vivo, by combined measurement of neuronal and hemodynamic parameters under both resting and stimulus evoked (activation) conditions. Under anaesthesia, the skull overlying somatosensory cortex was thinned to translucency for cortical imaging whilst burr holes were made to allow for the insertion of tissue oxygen or laser Doppler probes and recording or stimulating electrodes. Hemodynamic and neuronal data were acquired in cortical (barrel cortex) and subcortical structures under baseline and stimulation conditions, before and after the administration of 5-HT modulating drugs (or control substances), including agents in clinical use. Findings indicate a complex set of effects of serotonergic manipulations upon neurovascular function that depend upon not just specific receptor targets but also upon input pathways to the neuronal circuit under investigation. For instance, increasing serotonin release by intravenous administration of fenfluramine appeared to have minimal effects upon sensory (whisker) stimulation evoked changes in hemodynamics but more substantial effects upon direct cortical stimulation evoked CBF changes, suggesting an interaction of 5-HT modulation and neuronal pathway. Alterations in neurovascular coupling by changes in the function of the 5-HT system may have important implications for the application of functional MRI to investigate drug action. cognitive function, or disease mechanisms that involve 5-HT systems in both humans and animal research models.

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Poster

729. Neurovascular and Metabolic Coupling: Normal Brain and Stroke

Location: Halls B-H

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Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: Royal Society University Research Fellowship (CM)

Wellcome Trust Research Grant (CM)

Alzheimer's Research UK (KA & JB)

Title: Acute effects of systemic inflammation upon neurovascular coupling and neuroimaging signals

Authors: *G. BREZZO[1], J. SIMPSON[2], K. AMEEN-ALI[1], J. BERWICK[1], C. MARTIN[1]; [1]Psychology (Neuroscience), [2]Sheffield Inst. of Translational Neurosci. (SITraN), Univ. of Sheffield, Sheffield, United Kingdom

Abstract: Neuroinflammation is currently defined as any chronic or acute inflammatory process within the central nervous system and is a ubiquitous characteristic of many neurodegenerative diseases including Alzheimer's disease. The precise relationship between inflammatory processes and disease progression is complex and far from understood, but evidence from both animal and human studies suggests that in many cases inflammation may play in important role in the developing neuropathology. Inflammatory processes are mediated by the cells of the extended neurovascular unit, which includes neurons, astrocytes, pericytes, microglia and brain vascular cells. The neurovascular unit is simultaneously the substrate for the precise regulation of brain blood flow in accordance with local tissue requirements (neurovascular coupling). An important question therefore is whether and how the effects of inflammation on neurovascular unit function impact upon neurovascular coupling. Furthermore, it is important to determine how changes that occur at the cellular level in inflammation, for instance activation of glial cells, impact upon blood flow regulation in-vivo. To investigate this, we used an induced acute inflammation rodent model in which cerebral blood flow (CBF), neuronal activity and haemoglobin oxygenation and concentration were measured across a range of sensory stimulation parameters in order to quantify effects of inflammation on haemodynamic and neurovascular coupling measurements. In anaesthetised animals, a thin cranial window was prepared over the left somatosensory barrel cortex to enable recording of CBF using laser speckle contrast imaging as well as haemoglobin oxygenation and concentration with optical imaging spectroscopy. A surface electrode was placed adjacent to the thin window for neuronal activity recording. Data were acquired at two time intervals post lipopolysaccharide (LPS) or vehicle (saline) administration (2mg/kg i.p). Animal's brains were subsequently perfused and

extracted for immunohistochemistry to observe and quantify NVU and blood brain barrier changes including microglia, astrocytes, pericytes and ICAM-1/CD54 expression. This research has implications for understanding whether the effects of inflammation upon neurovascular coupling may constitute an early stage disease biomarker and/or a possible therapeutic target. Findings will also have implications for the interpretation of fMRI signals acquired from patients with neuroinflammation, where neurovascular coupling may be altered.

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Poster

729. Neurovascular and Metabolic Coupling: Normal Brain and Stroke

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Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

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Title: Serotonergic and noradrenergic modulation of neurovascular coupling in awake behaving mice

Authors: ***Q. ZHANG**[1], D. A. ANDERSON[1], K. W. GHERES[2], A. T. WINDER[1], P. J. DREW[1,3,4];
[1]Ctr. for Neural Engineering, Dept. of Engin. Sci. and Mechanics, [2]Grad. Program in Mol. Cell. and Integrative Biosci., [3]Dept. of Neurosurg., [4]Dept. of Biomed. Engin., The Pennsylvania State Univ., University Park, PA

Abstract: Hemodynamic signals are widely used to infer neural activity in functional brain imaging techniques, such as fMRI. Previous work (Huo, Smith and Drew, J. Neurosci., 2014) has shown that neural activity in the frontal cortex increases without corresponding changes in hemodynamic signals during locomotion, and we sought to understand the mechanism of this decoupling of neural activity from hemodynamic changes. Here, we investigated the effects of acute pharmacological manipulation of the serotonergic (5-HT) or noradrenergic systems on

neurovascular coupling by measuring neuronal and hemodynamic responses to voluntary locomotion in awake, head-fixed mice. Intrinsic optical signal imaging was used to measure cerebral blood volume (CBV) changes of frontal, visual, and somatosensory cortices through a polished thin-skull window, while neural activity (electrocortogram) was measured in the contralateral cortex concurrently. In a separate group of mice, local field potential and multiunit activity were measured in the frontal cortex and/or forelimb/hindlimb (FL/HL) representation in somatosensory cortex. Both neural activity and hemodynamic signals were collected before and after systemic administration of antagonists of 5-HT (trazodone), α1- (prazosin), α2-adrenoceptors (atipamezole), and vehicle controls. Compared to vehicle controls, neuronal responses to locomotion were not changed, CBV responses to locomotion were increased by both trazodone and prazosin in the frontal cortex and the FL/HL representation, and decreased by atipamezole in the FL/HL representation. These results suggest that in addition to local vasodilatory signals released from neurons and astrocytes, changes in neuromodulatory tone play an important vasoconstrictory role in shaping hemodynamic signals during behavior.

Disclosures: Q. Zhang: None. **D.A. Anderson:** None. **K.W. Gheres:** None. **A.T. Winder:** None. **P.J. Drew:** None.

Poster

729. Neurovascular and Metabolic Coupling: Normal Brain and Stroke

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 729.10/AAA12

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH Grant NS078168

NIH Grant NS079737

Scholar Award from the McKnight Endowment Fund for Neuroscience

National Scientist Development Grant 12DG9130022 American Heart Association

Title: Behavioral-state dependent inversion of neurovascular coupling in the somatosensory cortex of juvenile mice

Authors: *K. W. GHERES[1], C. J. ECHAGARRUGA[2], D. A. ANDERSON[3], P. J. DREW[1,4,5]; [1]Huck Inst. of the Life Sci., [2]Bioengineering, [3]Engin. Sci. and Mechanics, Pennsylvania State Univ. Univ. Park, University Park, PA; [4]Engin. Sci. and Mechanics, Pennnsylvania State Univ. Univ. Park, University Park, PA; [5]Neurosurg., Pennnsylvania State Univ. Hershey Med. Ctr., Hershey, PA

Abstract: In the adult somatosensory cortex, voluntary locomotion drives large increase in blood volume and blood flow (Huo, Smith, Drew, J. Neurosci., 2014). As neurovascular coupling can be weaker or inverted in the week or two following eye opening in rodents (Kozberg et al, 2013, PNAS), we sought to understand how neurovascular coupling in juvenile mice might be affected by behavior. Using two-photon microscopy, intrinsic optical signal (IOS) imaging and multielectrode arrays in awake mice, we found that neurovascular coupling under goes a behavioral-state dependent transformation during postnatal development. Stimulation of the vibrissae in the awake mouse caused biphasic (initial dilation followed by a constriction) hemodynamic response in juvenile mice that became adult-like within two weeks of eye-opening. Surprisingly, and in contrast to adults, voluntary locomotion drove a pronounced constriction and decrease in cerebral blood volume (CBV) in juvenile mice. Electrophysiological recordings show increases in spike rates and in the gamma band power of the field potentials power in the gamma band of the field potentials during locomotion in the somatosensory cortex, indicating inverted neurovascular coupling. This work suggests that state- and age-dependent changes in neurovascular coupling need to be taken into account in the interpretation of hemodynamic signals in the developing brain, but also provide insight into what signals might be integrated into the local hemodynamic response beyond local neural activity.

Disclosures: K.W. Gheres: None. **C.J. Echagarruga:** None. **D.A. Anderson:** None. **P.J. Drew:** None.

Poster

729. Neurovascular and Metabolic Coupling: Normal Brain and Stroke

Location: Halls B-H

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Program#/Poster#: 729.11/AAA13

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: ERC

Wellcome Trust

MRC

Rosetrees Trust

Title: What is an arteriole?

Authors: *M. R. HAMMOND-HALEY[1], D. AMIN[2], F. M. O'FARRELL[2], D. ATTWELL[2], C. HALL[3];
[1]Dept. of Med., BSMS, The City of Brighton and Hove, United Kingdom; [2]Dept. of

Neuroscience, Physiol. and Pharmacol., UCL, London, United Kingdom; [3]Sussex Neurosci., Univ. of Sussex, Brighton, United Kingdom

Abstract: Neurovascular coupling is classically thought to be mediated by annular smooth muscle cells around arterioles. Capillaries, in contrast, are traditionally considered to be non-contractile. However, vascular mural cells which extend processes along and around small (4μm) vessels also regulate blood flow (1, 2). These cells are classically considered to be pericytes, and the vessels they lie on capillaries. Recently, however, it has been suggested that these contractile cells are smooth muscle cells, despite their gross difference in morphology from arteriolar smooth muscle, and their position on small vessels (2). We investigated where, along the arteriole-capillary axis, different vascular functions transition from “arteriole-like” to “capillary-like”.

We used genetic and immunohistochemical labelling for markers of different cerebrovascular functions, to relate vascular function to morphology of vascular mural cells and their position in the vascular tree. Slices of adult NG2-DsRed mouse forebrain were labelled with antibodies or fluorescent dyes against PDGFRβ, α-SMA, nestin, GLUT-1 and elastin. For each protein, the point of transition in labelling (if any) along the arteriole-capillary axis was compared with branching order, vessel diameter and mural cell inter-soma distance (an indicator of vascular mural cell morphology).

There was a gradual transition of functions between arterioles and capillaries. Unlike the classical view, nutrient transport and BBB regulation is supported throughout the vascular tree, as shown by expression of the glucose transporter GLUT-1 and BBB-regulator PDGFRβ throughout the cerebrovasculature. Contractile function gradually changes across the vascular bed, with α-SMA being expressed further along the vascular tree than elastin, an arteriole marker. Finally, angiogenic potential, as indicated by expression of nestin, a marker of proliferative potential, is expressed in the capillary bed, in vessels that also express alpha-SMA and are significantly smaller than those expressing elastin.

Appreciating the heterogeneity of functions along the vascular tree will be crucial for understanding how the cerebrovasculature functions in health and disease.

1.Hall CN, Reynell C, Gesslein B, Hamilton NB, Mishra A, Sutherland BA, et al. Capillary pericytes regulate cerebral blood flow in health and disease. Nature. 2014 Apr 3;508(7494):55-60.

2.Hill RA, Tong L, Yuan P, Murikinati S, Gupta S, Grutzendler J. Regional Blood Flow in the Normal and Ischemic Brain Is Controlled by Arteriolar Smooth Muscle Cell Contractility and Not by Capillary Pericytes. Neuron. 2015 Jul 1;87(1):95-110.

Disclosures: M.R. Hammond-Haley: None. **D. Amin:** None. **F.M. O'Farrell:** None. **D. Attwell:** None. **C. Hall:** None.

Poster

729. Neurovascular and Metabolic Coupling: Normal Brain and Stroke

Location: Halls B-H

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Program#/Poster#: 729.12/AAA14

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH R01HL113863-04

NIH 5T32MH019113-23

VA IBX000741B

NARSAD

Title: Acid-sensing ion channel-1A regulates CO_2-induced vasodilation

Authors: *R. J. TAUGHER[1], C. M. LYNCH[2], S. C. GUPTA[1], R. FAN[1], F. M. FARACI[2], J. A. WEMMIE[1];
[1]Psychiatry, [2]Intrnl. Med., Univ. of Iowa, Iowa City, IA

Abstract: Regulation of cerebral blood flow (CBF) is critical for normal brain function, and insufficient CBF may contribute to cognitive impairments in neurological conditions such as dementia. Though the regulation of CBF is complex and multifaceted, acidosis, such as that induced by carbon dioxide (CO_2), is a strong regulator of CBF. Nitric oxide (NO) production is thought to be critical for CO_2-induced vasodilation; however, the mechanisms by which CO_2 influences NO production remain unclear. Acid-sensing ion channel-1A (ASIC1A) is a proton-gated cation channel that is activated by extracellular acidosis. Previous work has implicated ASIC1A in the amygdala and bed nucleus of the stria terminalis in CO_2- and acid-evoked behavior. Thus, we hypothesized that ASIC1A might also contribute to the microvascular response to CO_2 and acidosis. To test this hypothesis we genetically and pharmacologically manipulated ASIC1A in mice and assessed the effects on CO_2-induced vasodilation. We found that the effect of CO_2 on vessel diameter was greatly attenuated in *Asic1a*$^{-/-}$ mice and in mice treated with the ASIC inhibitor, psalmotoxin. These manipulations did not alter the vasodilatory effects of acetylcholine, suggesting that endothelial NO production was unaffected, and further suggesting a neuronal source of NO. Thus, we hypothesized that neurons might be the site of ASIC1A action in the microvascular response to CO_2 and we generated mice in which *Asic1a* is specifically disrupted in neurons. We found that the effect of CO_2 on vessel diameter was also attenuated in these mice. To further explore a role for ASIC1A in the regulation of NO production, we measured NO concentrations in whole brain lysates immediately after exposure to CO_2. We found that 10% CO_2 induced NO production in wild-type mice, but not in mice lacking *Asic1a* globally or specifically in neurons. Together, these data are consistent with a

model wherein activation of neuronal ASIC1A by hypercarbic acidosis is critical for CO_2-induced vasodilation and NO production. These studies raise the possibility that ASIC1A may play a critical role in regulating CBF in response to other sources of acidosis, including neuronal activity, metabolism, and pathophysiologic conditions such as seizure and ischemic stroke.

Disclosures: R.J. Taugher: None. **C.M. Lynch:** None. **S.C. Gupta:** None. **R. Fan:** None. **F.M. Faraci:** None. **J.A. Wemmie:** None.

Poster

729. Neurovascular and Metabolic Coupling: Normal Brain and Stroke

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Support: NSF Grant BCS 41446377

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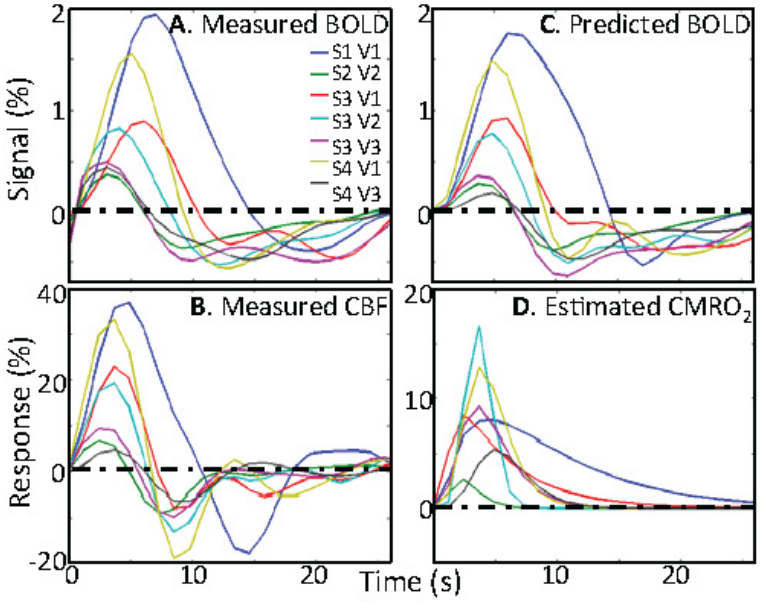
NIH Grant HL108143

Title: Time-resolved measurements of human cerebral oxygen metabolism using magnetic resonance imaging

Authors: *D. RESS, J. KIM;
Neurosci., Baylor Col. of Med., Houston, TX

Abstract: Brief neural activation creates a hemodynamic response function (HRF), a stereotypic manifestation of neurovascular coupling. A recent theory (Kim & Ress, Neuroimage, 2016) predicts that the HRF is created by a combination of flow and cerebral oxygen metabolism ($CMRO_2$). Here, we used arterial spin-labeling (ASL) to measure both the HRF and flow, then used our model to obtain estimates of the $CMRO_2$ time course. **Methods**: Subjects ($N = 4$) view a 2-s duration audiovisual stimulus while performing a speeded task. Stimuli were separated by a 30-s interstimulus interval, and repeated 16 times per run for 5 runs to create a total of 80 measurements per session. Combined flow and HRF data were obtained using a pseudo-continuous ASL sequence on a Siemens 3T scanner with 2-mm voxels and TR = 3 s on 13 quasi-axial slices. Stimulus presentations were jittered to increase temporal sampling to 1.5 s. The flow and HRF data were transformed into a high-resolution (0.7-mm) reference anatomy so that they could be averaged within visual areas V1—3, predefined for each subject. The model was then used to estimate $CMRO_2$ time course from the data assuming a gamma-function temporal form. **Results**: All subjects show a significant flow undershoot (Figure), and some show a more

complex oscillatory return to baseline. Standard errors were <5% of peak amplitude for all measurements. Flow response peaks ~4 s earlier than the BOLD HRF. Most $CMRO_2$ estimates are prompt, but 2—4 measurements show a slow return to baseline. **Discussion**: The flow induced by brief stimulation has a strong undershoot and complex late time behavior consistent with underdamped oscillation. HRF responses are substantially delayed from the flow response, consistent with convective time delays as the flow propagates into downstream venous microvasculature that dominates BOLD contrast. The estimated $CMRO_2$ time course suggests that metabolism usually returns to baseline fairly rapidly, but metabolism can sometimes persist long after the activation.



CBF and HRF measurements and modeling. **A)** *Measured HRFs in 4 subjects and 3 visual areas.* **B)** *Measured flow.* **C)** *BOLD predictions from the model.* **D)** *Estimated $CMRO_2$ from the model*

Disclosures: D. Ress: None. **J. Kim:** None.

Poster

729. Neurovascular and Metabolic Coupling: Normal Brain and Stroke

Location: Halls B-H

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Program#/Poster#: 729.14/AAA16

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: 09SDG2060701

NS37853

Title: Obligatory role of EP-1 receptors in the increases in cerebral blood flow produced by hypercapnia in the mouse brain microcirculation.

Authors: *K. UEKAWA, P. ZHOU, N. BRUNIER, Y. HATTORI, C. IADECOLA, L. PARK; Feil Family Brain & Mind Reseach Inst., Weill Cornell Med. Col., New York, NY

Abstract: Hypercapnia is a potent vasodilator stimulus in the cerebral circulation. Although it has long been known that prostanoids participate in the cerebrovascular effects of hypercapnia (JCBFM, 14:175-192, 1994), the role of prostaglandin E2 (PGE2) and PGE2 receptors have not been fully investigated. We sought to determine whether cyclooxygenase-1 (COX-1)-derived PGE2 and EP1 receptors are involved in the cerebrovascular response induced by hypercapnia. Male EP1-/- mice and wild type (WT) littermates (age 3-4 months; n=5/group) were anesthetized with urethane-chloralose and equipped with a cranial window overlying the somatosensory cortex. Cerebral blood flow (CBF) was recorded by laser-Doppler flowmetry with controlled blood pressure and physiological variables. In WT mice, neocortical superfusion of the EP1 receptor antagonist SC-51089 (10 μM) attenuated the increase in CBF elicited by systemic hypercapnia (pCO_2=50-60 mmHg) by 41% (CBF increase: vehicle, 69±5%; SC-51089, 41±3%; $p<0.05$). SC-51089 also attenuated the increase in CBF produced by neocortical application of arachidonic acid (AA: 10μM) (AA, 21±5%; AA+SC51089, 9±3%; -56%; $p<0.05$) and PGE2 (5 μM) (PGE2, 16±2%; PGE2+SC51089, 9±2%; -38%; $p<0.05$). These responses were also attenuated in EP1-/- mice. In contrast, in WT mice treated with SC-51089 or in EP1-/- mice the CBF increase elicited by neocortical application of the endothelium-dependent vasodilator acetylcholine (ACh: WT, 24±2%; WT+SC-51089, 23±2%; EP1-/-, 23±2%; $p>0.05$) or the smooth muscle relaxant adenosine (WT, 33±2%; WT+SC-51089, 31±1%; EP1-/-, 31±2%; $p>0.05$) were not affected. Similarly, the CBF increase evoked by whisker stimulation (functional hyperemia) (WS: WT, 24±1%; WT+SC-51089, 23±1%; EP1-/-, 24±2%; $p>0.05$) was not attenuated. In WT mice, the COX-1 inhibitor SC-560 (25μM), but not the COX-2 inhibitor NS-398 (100 μM), attenuated the hypercapnic CBF increase by 50% (vehicle, 62±5%; SC-560, 31±5%; $p<0.05$). Neocortical application of PGE2 (1 μM) did not affect resting CBF ($p>0.05$), but counteracted the attenuation of the hypercapnic response induced by SC-560 (SC-560, 44±4%; SC-560+PGE2, 66±6%; $p<0.05$). In contrast, exogenous PGE2 did not rescue the attenuation induced by SC-51089 (SC-51089, 39±4%; SC-51089+PGE2, 43±5%; $p>0.05$), attesting to the obligatory role of EP1 receptors in the response. The findings indicate that the hypercapnic vasodilatation depends on COX-1-derived PGE2 acting on EP-1 receptors and highlight the critical role that COX-1 derived prostanoids and EP1 receptors play in the regulation of the cerebral circulation.

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Poster

729. Neurovascular and Metabolic Coupling: Normal Brain and Stroke

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 729.15/AAA17

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Title: Imaging of cerebral blood flow in rodent models with SPECT, MRI and autoradiography

Authors: J. RYTKÖNEN, K. LEHTIMÄKI, A. SHATILLO, L. TOLPPANEN, *T. T. AHTONIEMI, A. NURMI, T. HUHTALA;
Charles River Discovery, Kuopio, Finland

Abstract: Regulation of blood flow and oxygen delivery in the brain is essential for survival. In a normal physiological state the cerebral blood flow (CBF) stays very constant due to persistent adjustment of vascular resistance according to blood pressure. Imaging of CBF has clinical relevance as functional abnormalities can signal pathophysiological changes e.g. stroke and neurodegenerative diseases. In this study methods to measure CBF in rodent models are presented. Single photon emission computed tomography (SPECT) imaging after intravenous injection of ^{99m}Tc-exametazime (^{99m}Tc-HMPAO) is a conventional method to asses CBF *in vivo*. It has been shown to correlate strongly with regional brain perfusion and is used in clinical nuclear imaging to detect stroke and other cerebrovascular diseases. Arterial spin labeling allows capturing of brain perfusion with magnetic resonance imaging (MRI). It is a noninvasive method where protons in arterial blood are magnetically labeled and subsequently imaged in the region of interest. Therefore, no injection of contrast agent is needed to obtain information about cerebral perfusion. Arterial spin labeling MRI sequences are increasingly being used in clinical imaging to provide quantification of CBF. Although ^{99m}Tc-HMPAO-SPECT and arterial spin labeling MRI techniques are translational between clinical and pre-clinical studies, differences arise in the required use of anesthesia. One of the major challenges to study neurological function in rodents is due to use of restriction, anesthesia or paralyzing agents. All of these methods have impact to brain perfusion e.g. altering the vasoconstriction and blood pressure. To study immediate neuronal effect, delivery of radiotracer that reaches a cerebral equilibrium in a short time frame will allow imaging with great temporal resolution. ^{14}C-iodoantipyrine has shown a strict linear proportionality between tissue radioactivity and CBF when the data is captured within a brief interval after the tracer injection. As an case example to study CBF without restrain or anesthesia in mice, the animals were cannulated in jugularis vein week prior to administration of radiotracer. Tracer was injected trough cannula connected to a tether, followed by immediate dosing of euthanizing solution. Brains were collected and quantified using autoradiography. Alterations in CBF between studied brain regions were quantified. As a summary, several translational approaches can be applied in rodent models to monitor CBF alterations associated to disease progression or drug effect.

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Poster

729. Neurovascular and Metabolic Coupling: Normal Brain and Stroke

Location: Halls B-H

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Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: Chonbuk National University, Clinical Trial Center

Title: Ethanolic extracts from Morus alba L. fruit induce vasorelaxation of the rat aortic smooth muscle

Authors: H. LEE[1], S. PARK[2], Y. BAE[2], *J. KIM[3], S. YOON[4], S. HAHN[4], H.-J. CHAE[1], S.-W. CHAE[1], B. CHOI[1];
[1]Pharmacol., Inst. for Med. Sci., Jeonju, Korea, Republic of; [2]Physiol., Konkuk Univ. Sch. of Med., Seoul, Korea, Republic of; [3]Med. Col. of Georgia, Augusta Univ., Augusta, GA; [4]Physiol., Col. of Medicine, The Catholic Univ. of Korea, Seoul, Korea, Republic of

Abstract: *Morus alba* L., or mulberry, has traditionally been known to induce antibacterial, antioxidant, and hypolipidemic effects. Recent evidence suggests that an extract of *Morus alba* L. decreases the high blood pressure in diabetic rats. It is unknown whether a *Morus alba* L. extract affects the contraction of vascular smooth muscle. In this study, the aqueous and ethanolic extracts of *Morus alba* L. fruit were evaluated for vasorelaxation of the rat aorta. The endothelium-intact aortic smooth muscle was isolated from 10-week-old Sprague-Dawley rats and its contractile force was measured on a tension transducer. The intactness of the endothelium was confirmed by acetylcholine-induced relaxation. The ethanolic extract (with 40% ethanol) of *Morus alba* L. contained a higher level of cyanidin-3-glucoside than the aqueous extract: 1994 ± 93 mg/100 g and 1746 ± 98 mg/100 g, respectively. To assay the vasorelaxant effect of *Morus alba* L. extracts, the aortic rings were first contracted by serotonin (5-HT), high K^+ (70 mM) and norepinephrine (NE) and then the *Morus alba* L. extracts were applied. The aqueous extracts of *Morus alba* L. did not induce vasorelaxation of aortic rings that were pre-contracted by 5-HT, high K^+ and NE. However, the ethanolic extracts caused vasorelaxation of aortic rings that were pre-contracted by 5-HT or high K^+, but not NE. This study demonstrates that an extract of *Morus alba* L. fruit might act as a vasorelaxant for the aortic smooth muscle. The lack of the effect on NE-treated aorta implies that the vasorelaxation effect of *Morus alba* L. might be mediated by specific signaling cascades, e.g., related to 5-HT or high K^+. The identity of the active ingredient(s) and the underlying mechanisms need to be further determined.

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Poster

729. Neurovascular and Metabolic Coupling: Normal Brain and Stroke

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 729.17/AAA19

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Title: Probing the link between indolent aneurysmal dome infections and brain aneurysm growth and rupture

Authors: ***J. KELLY**[1], T. ST.AMAND[1], J. KROL[2], G. D. EHRLICH[2], D. J. COOK[1]; [1]Queen's Univ., Kingston, ON, Canada; [2]Drexel Univ., Philadelphia, PA

Abstract: *Background*: Vascular inflammation is thought to play an important role in intracranial aneurysm (IA) formation and rupture. Recent studies have shown potential bacterial etiology behind this inflammation. Dental and respiratory microbial DNA has been discovered in unruptured and ruptured aneurysms from patients with no clinically diagnosable infection. However, more comprehensive analyses are required before this interaction can be understood. To this end, we explored the possibility that IA progression is related to indolent, subclinical infection of the aneurysm dome by microorganisms originating from commensal or pathogenic populations existing elsewhere in the host. *Methods*: IA dome samples were collected surgically from patients undergoing IA clippings (7 of each stable, growing and ruptured). Total genomic DNA was extracted from each sample for analysis using the Ibis T5000 Biosensor, allowing for species-specific identification of the microorganisms present. Positive biosensor results were confirmed through sequencing using an Illumina MySeq. Microbial population results were pooled for each sample type and compared amongst each other. Further, pooled results were compared against known commensal and pathogenic populations from elsewhere in the body to deduce the likely source of the organisms. *Results and Discussion*: Populations of commensal organisms were found associated with stable, growing and ruptured aneurysm tested. While results pertaining to microbial etiology of IA progression remain inconclusive, we have successfully provided a metagenomic catalogue of organisms that may be associated with IAs along with their likely point of origin.

Disclosures: **J. Kelly:** None. **T. St.Amand:** None. **J. Krol:** None. **G.D. Ehrlich:** None. **D.J. Cook:** None.

Poster

729. Neurovascular and Metabolic Coupling: Normal Brain and Stroke

Location: Halls B-H

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Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: Research Manitoba

JG Fletcher PhD Fellowship in Functional Foods and Nutraceuticals

Title: Effect of stilbenoid polyphenols on aberrant cerebral artery morphology and mechanics in the spontaneously hypertensive heart-failure (SHHF) rat

Authors: *C. ACOSTA[1,2], H. ANDERSON[1,3], D. LEE[1,3], C. ANDERSON[4,2];
[1]St. Boniface Hosp. Albrechtsen Res. Ctr., Winnipeg, MB, Canada; [2]Pharmacol. & Therapeut., [3]Col. of Pharm., Univ. of Manitoba, Winnipeg, MB, Canada; [4]Kleysen Inst. for Advanced Med., Winnipeg, MB, Canada

Abstract: Hypertension is an important risk factor for cognitive decline and dementia. Further adding risk of heart failure *per se* then cumulatively increases the possibility of cognitive decline and promotes progression to *bona fide* dementia. This may be due, at least in part, to altered morphology and mechanical properties of cerebral arteries and arterioles. We hypothesized that hypertension combined with added risk of heart failure (as found in the SHHF rat) confer morphological and mechanical aberrations in middle cerebral arteries. Resveratrol (trans-3,5,4'-trihydroxystilbene) is a stilbenoid polyphenol that is purportedly linked to improved longevity, cardiovascular health, and benefits in Alzheimer's disease. Although resveratrol is well-tolerated in humans, it is readily metabolized and exhibits low bioavailability. Thus, we also queried whether resveratrol analogues would exert greater vasculoprotective effects. Sprague-Dawley (SD) and SHHF rats (n=8) were treated for 8 weeks by gavage with vehicle control (C) or low doses (2.5 mg/kg/d) of resveratrol (R), pterostilbene (P), and gnetol (G). Blood pressure (BP) was measured by tail-cuff plethysmography. Vascular geometry and mechanical properties of isolated middle cerebral arteries were measured by pressure myography. Systolic BP increased in the SHHF rat (196±3 mm Hg vs. SD 142±7 mm Hg, $p<0.01$), and was unaffected by stilbenoid treatment. SHHF arteries exhibited increased media-to-lumen ratios (15.9±2.4 vs. SD 9.0±0.5, *$p<0.05$*), and were associated with remodelling and growth indices of 58% and 44%, respectively. All three stilbenoids reduced media-to-lumen ratios (SHHF-R 12.3±1.7, SHHF-P 12.8±1.1, SHHF-G 13.2±1.6, *$p<0.01$*). Wall component stiffness (i.e. slope of elastic modulus *vs*. stress) was augmented in SHHF (18.2±2.9 vs. SD 6.1±0.9, *$p<0.01$*), and was attenuated in part by resveratrol and gnetol (SHHF-R 11.8±1.1, SHHF-G 11.5±1.0, *$p<0.05$ vs. SD and $p<0.01$ vs. SHHF-C*) and completely by pterostilbene (SHHF-P 9.4±1.1, *not significant vs. SD and $p<0.0001$ vs. SHHF-C).* In summary, SHHF middle cerebral arteries exhibited a combination of

eutrophic and hypertrophic remodeling. Stilbenoids improved vascular morphology and geometry-independent stiffness independently of blood pressure lowering, suggesting direct actions on the arterial wall. The ability of pterostilbene to entirely normalize wall component stiffness may be related to its reported superior bioavailability. We conclude that further research is warranted on stilbenoid polyphenols as an adjunct to current cardiovascular treatment regimens that might reduce the risk of cognitive decline.

Disclosures: **C. Acosta:** None. **H. Anderson:** None. **D. Lee:** None. **C. Anderson:** None.

Poster

729. Neurovascular and Metabolic Coupling: Normal Brain and Stroke

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 729.19/AAA21

Topic: C.08.Stroke

Title: Individual patterns of cognitive impairment after transient ischemic attack

Authors: *L. E. SIMMATIS[1], S. H. SCOTT[1], A. Y. JIN[2];
[1]Ctr. For Neurosci. Studies, [2]Med., Queen's Univ., Kingston, ON, Canada

Abstract: Transient ischaemic attack (TIA) is a major indicator of impending stroke risk. There is evidence that TIA patients display chronic impairment which may persist for days to months after the event. The underlying cognitive and motor deficits which contribute to this chronic impairment have not been elucidated. We assessed a group of 23 TIA patients using both subjective clinical scoring systems and quantitative tools. We administered the Purdue pegboard test (PPB) to test fine motor control, the Chedoke-McMaster Stroke Assessment (CMSA) to determine gross motor impairment stage, the Behavioural Inattention Test (BIT) to test visual neglect, and the Montreal Cognitive Assessment test (MoCA) to detect mild cognitive impairment. We then performed kinematic analysis using the bimanual KINARM robot, which has been previously validated as a reliable tool for studying stroke patients. The battery of 8 KINARM tasks that we used spanned motor, sensory, and cognitive functional domains. We normalized all kinematic data to a healthy control population of over 100 individuals correcting for the influence of age, gender, and handedness in order to derive Z-scores for both parameter-level and task-level performance.
BIT, PPB and MoCA identified a few TIA subjects as impaired (n=5, 5, and 6) and CMSA scores deviated from the ideal healthy range for several subjects (n=8 patients with a score of 5 in at least one component). The robot-based analysis found that motor and sensory tasks were less impacted than cognitive ones. The ability to complete simple motor tasks involving reaching and object hitting was affected in some patients (n=3), as was proprioceptive upper limb sense

(n=5). However, 39% and 30% of our cohort were impaired in spatial working memory and visuomotor integration. There was a broad range of different patterns of impairments across subjects. These results demonstrate that TIA patients show individual patterns of impairment which tend to be biased towards the cognitive domain. Furthermore, cognitive impairments were not detected using traditional clinical assessment tools. This is possibly due to a lack of sensitivity of these tools to the types of mild impairment which are present after a TIA. This work highlights the need for individual consideration of TIA patients on a case-by-case basis and the need for awareness of 'silent' symptoms which may not be readily detectable in the clinic, but may provide functional difficulties at later time points after the event.

Disclosures: L.E. Simmatis: None. **S.H. Scott:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); SHS is the cofounder and chief scientific officer of BKIN Technologies, the company that commercializes the robotic technology used in this study. **A.Y. Jin:** None.

Poster

729. Neurovascular and Metabolic Coupling: Normal Brain and Stroke

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 729.20/AAA22

Topic: C.08.Stroke

Support: Canadian Partnership for Stroke Recovery

Title: White matter hyperintensities in neurologically intact aging individuals impact arm function

Authors: A. M. AURIAT[1], J. K. FERRIS[1], S. E. BLACK[2], *L. A. BOYD[3];
[1]Physical Therapy, Univ. of British Columbia, Vancouver, BC, Canada; [2]Sunnybrook, Toronto, ON, Canada; [3]Univ. British Columbia, Vancouver, BC, Canada

Abstract: Introduction: White matter hyperintensities (WMH) are frequent in aging individuals and are known to affect cognition, balance, and gait. However, the affects of WMHs on upper-limb performance are unknown.
Methods: The KINARM robot was used for 4 upper-limb assessments. 1) Visually Guided Reaching: participants moved as quickly as possible to a target. 2) Arm Matching: the robot moved participants' hand to a target positions and the participant had to mirror match the location with the opposite hand. 3) Object-Hit: participants hit virtual balls moving towards them with virtual paddles attached to each hand. 4) Object-Hit and Avoid: cognitive demand of the Object-Hit task was increased, by requiring participants to hit two target shapes while avoiding

all others. Semi-automatic volumetric assessment of covert lesions was completed with magnetic resonance imaging (MRI).
Results: We assessed 26 normally aging individuals (range: 43-80; mean ± SD: 61.48 ± 9.88). Accuracy of visually guided reaching did not relate to the volume of WMHs ($p \geq 0.117$), but higher WMH volume related to increased movement time for dominant and non-dominant arms ($r = 0.542, 0.479$; $p \leq 0.018$). Arm Matching and Object-Hit performance did not relate to WMH volume ($p > 0.05$). However, on Object-Hit and Avoid, WMH volume correlated with median error ($r = -0.574$), hand bias of hits ($r = 0.564$), and hand transition ($r = -0.530$; $p \leq 0.008$).
Conclusion: Individuals with more WMHs took longer to complete target reaching, however, this did not affect accuracy. When difficulty and cognitive demand increased WMHs significantly impaired performance. Specifically, on a bimanual task, individuals with higher levels of WMH shifted limb-use to favor the dominant limb, with a resulting decrement in performance. These findings suggest an important link between WMHs and attenuated motor performance in normally aging individuals.

Disclosures: **A.M. Auriat:** None. **J.K. Ferris:** None. **S.E. Black:** None. **L.A. Boyd:** None.

Poster

729. Neurovascular and Metabolic Coupling: Normal Brain and Stroke

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Topic: C.08.Stroke

Support: NIH Grant R01 NS078026

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Title: 20-HETE inhibition protects against intracerebral hemorrhage injury

Authors: ***X. HAN**[1], X. ZHAO[1], X. LAN[1], Y. GAO[1], Z. YANG[1], J. FALCK[2], R. KOEHLER[1], F. GUAN[3], J. WANG[1];
[1]Johns Hopkins Hosp., Baltimore, MD; [2]Univ. of Texas Southwestern Med. Ctr., Dallas, TX; [3]zhengzhou university, zhengzhou, China

Abstract: Background and Purpose: Intracerebral hemorrhage (ICH) accounts for 15% of all strokes. Previous studies have revealed that 20-HETE, a metabolite of arachidonic acid

synthesized by cytochrome P450 4A, plays an important role in ischemic stroke injury and subarachnoid hemorrhage. However, little is known about the role of 20-HETE after ICH. In this study, we examined the hypotheses that 20-HETE inhibition has a protective effect after collagenase-induced ICH injury and that it affects angiogenesis. **Methods**: To investigate the protective effect of 20-HETE synthesis inhibitor N-hydroxy-N'-(4-butyl-2-methylphenyl)-formamidine (HET0016) after ICH, we exposed hippocampal slice cultures to hemoglobin (10 μM) and induced ICH in mice by intrastriatal collagenase injection. Hemoglobin-induced neuronal death was assessed by propidium iodide (PI) after 18 hours in vitro. Lesion volume, neurologic deficits, cell death, reactive oxygen species (ROS) production, neuroinflammation, and angiogenesis were evaluated in mice at different time points after ICH. **Results:** In cultured mouse hippocampal slices, 5, 14-HEDGE, a 20-HETE mimetic, exacerbated hemoglobin-induced neuronal cytotoxicity in the CA1 area, whereas HET0016 treatment attenuated hemoglobin-induced neuronal damage and decreased levels of proinflammatory cytokines and reactive nitrogen species (n=25 slices/group, $p<0.05$). In vivo, HET0016 treatment reduced brain lesion volume and neurologic deficits, and decreased neural death, ROS production, matrix metalloproteinase gelatinolytic activity, and the inflammatory response at 3 days after ICH (n=8/group, $p<0.05$). Src kinase phosphorylation was increased after ICH and decreased by HET0016 treatment (n=8/group, $p<0.01$). However, HET0016 did not affect angiogenesis, as assessed by VEGF, VEGFR2, and CD31 levels on day 28 after ICH (n=6/group*)*. **Conclusion:** These findings suggest that 20-HETE plays an important role in ICH-induced brain damage. Modulating the 20-HETE signaling pathway may provide a viable means to mitigate ICH impairment without angiogenesis inhibition.

Disclosures: X. Han: None. **X. zhao:** None. **X. lan:** None. **Y. gao:** None. **Z. yang:** None. **J. Falck:** None. **R. Koehler:** None. **F. guan:** None. **J. wang:** None.

Poster

729. Neurovascular and Metabolic Coupling: Normal Brain and Stroke

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 729.22/AAA24

Topic: C.09. Brain Injury and Trauma

Support: NIH NS082957

NIH NS083007

Title: Controlling oxidative stress promotes functional recovery after TBI

Authors: *R. VEMUGANTI[1], S. MEHTA[1], T. KIM[1], R. CHANDRAN[1], E. UDHO[2], P. CENGIZ[2], R. DEMPSEY[1];
[1]Neurolog. Surgery, [2]Pediatrics Waisman Ctr., Univ. of Wisconsin, Madison, WI

Abstract: The secondary neuronal death is a known proponent of long-term neurological dysfunction following traumatic brain injury (TBI). Oxidative stress after TBI kills neurons if uncontrolled. Paradoxically, pathways that promote as well as fight the oxidative stress are induced concurrently after TBI. NADPH oxidase subunit NOX2 promotes formation of reactive oxygen species that kills neurons. On contrary, activation of transcription factor Nrf2 promotes the expression of down-stream neuroprotective genes including anti-oxidant enzymes and protein chaperones. We currently evaluated if inhibiting the pro-oxidant NOX2 and/or potentiating the anti-oxidant Nrf2 individually or in combination decreases the motor and cognitive deficits and secondary cortical contusion after TBI. Controlled cortical impact-induced TBI (mild to moderate) in adult mice significantly induced the expression of both NOX2 and Nrf2 in the ipsilateral cerebral cortex at 6h and 24h after injury. Cohorts of mice subjected to TBI were treated with either an inhibitor of NOX2 (apocynin; 10 mg/kg) or an activator of Nrf2 (TBHQ; 25 mg/kg) or both or vehicle. We tested the drug administration at 2 time schedules (each drug given twice; either at 5 min and 24h or at 2h and 24h after TBI). Mice were subjected to motor testing (rotarod test and beam walk test) between days 1 and 7 and Morris water maze test between days 26 to 30 after TBI. Treatment with either apocynin or TBHQ alone significantly improved the motor function after TBI compared to vehicle control. The drug treated mice stayed longer on rotarod and made fewer foot faults in beam walk test. The combination therapy also significantly improved the post-TBI motor function recovery compared to vehicle control, but there was no additive effect of the 2 drugs. The mice treated with the combination of apocynin and TBHQ also performed better in the Morris Water maze test. Animals in the drug groups spent significantly greater time with higher frequency in the platform quadrant as compared to vehicle group. All mice were sacrificed and the secondary cortical contusion was estimated using the serial brain sections stained with Cresyl violet. The cortical contusion volume was not different in either apocynin or TBHQ alone groups compared to the vehicle control group. However, the combo therapy (apocynin+ TBHQ) given either at 5 min/24h or 2h/24h led to a significantly smaller cortical contusion volume compared to vehicle control group. Thus, these studies show that controlling oxidative stress is a potential therapy to minimize post-TBI motor and cognitive deficits and secondary brain damage. Funded by NIH.

Disclosures: **R. Vemuganti:** A. Employment/Salary (full or part-time): University of Wisconsin, Madison, William S. Middleton VA Hospital. **S. Mehta:** A. Employment/Salary (full or part-time): University of Wisconsin, Madison. **T. Kim:** None. **R. Chandran:** None. **E. udho:** None. **P. Cengiz:** A. Employment/Salary (full or part-time): University of Wisconsin, Madison. **R. Dempsey:** A. Employment/Salary (full or part-time): University of Wisconsin, Madison.

Poster

729. Neurovascular and Metabolic Coupling: Normal Brain and Stroke

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 729.23/AAA25

Topic: C.08.Stroke

Support: NIH/NINDS, R01NS060768

NIH/NINDS, R01NS064109

Title: HO-1 inhibition delay hematoma cleanup after intracerebral hemorrhage

Authors: L. ZHANG[1], X. ZHAO[2], G. SUN[2], *J. ARONOWSKI[2];
[1]Neurol., Beijing Friendship Hospital, Capital Med. Univ., Beijing, China; [2]Neurol., Univ. Texas HSC - Houston, Houston, TX

Abstract: The red blood cells (**RBC**) and their hemolytic products (including hemoglobin, heme, and iron) which represent main components of brain hematoma after intracerebral hemorrhage (**ICH**), trigger a series of adverse biochemical events leading to secondary brain injury and neurological deficits. The efficient removal of the hematoma components is necessary for achieving inflammation resolution and functional recovery. The inducible heme-oxygenase (**HO-1**) is a key rate-limiting enzyme that catabolizes heme into iron, carbon monoxide, and biliverbin. The present study investigated the spatial and temporal expression of HO-1, as well as its possible function in the hematoma absorption. We found that HO-1 after ICH was upregulated around the hematoma area starting from 6h, reaching the maximum level at 3 - 7days, and persisting for at least 10 days after ICH. HO-1 immunohistochemistry shows that the most HO-1-positive cells are CD68-positive microglia/macrophages (**MΦ**). To explore the pathophysiological role of HO-1 after ICH, a competitive HO inhibitor, tin-protoporphyrin IX (**SnPP**, 7.5 mg/kg), was injected, ip, twice a day, for 7 days. SnPP significantly delayed hematoma clearance by 27.8% and significantly impaired the functional recovery as measured at day 7 after ICH. Histological analyses showed that there are more TUNEL-positive neurons at the hematoma-affected brain tissue in SnPP-treated brains. In addition, we isolated and cultured the rat brain microglia (MΦ). Upon exposing to RBC, MΦ phagocytize/digest the RBC, and HO-1 is induced during this process. Co-incubating 10 μM SnPP with RBC significantly delayed RBC internalization by MΦ. Subjecting primary rat brain neuron-microglia co-cultures to RBC and mild (sublethal) oxygen-deprivation (an ICH-like injury) caused neuronal damage, as measured by neurofilament degradation. Adding SnPP further aggravated the neuronal death. We postulate that HO-1 is important for MΦ-mediated brain hematoma clearance after ICH.

Disclosures: L. Zhang: None. **X. Zhao:** None. **G. Sun:** None. **J. Aronowski:** None.

Poster

729. Neurovascular and Metabolic Coupling: Normal Brain and Stroke

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 729.24/AAA26

Topic: C.08.Stroke

Support: MOST Grant MOST-104-2320-B-001-006-MY3

Title: Clinacanthus nutans ameliorated neuronal apoptosis and ischemic brain injury by downregulating HDAC1/6

Authors: *J.-S. WU, H.-D. TSAI, M.-H. KAO, W.-M. CHEUNG, T.-N. LIN;
Neurosci. Division, IBMS Academia Sinica, Taipei, Taiwan

Abstract: BACKGROUND AND OBJECTIVE:
Growing evidence supports epigenetic regulation in the pathogenesis and recovery of stroke. Histone deacetylases (HDACs) inhibition has been shown to confer anti-inflammatory and neuroprotective effects. C. nutans is a folk medicine in Southern Asia for infection, inflammation, cancer, and Diabetes treatment. But, there is no data in treating ischemic stroke yet. In this study, we aimed to investigate whether C. nutans protects brain against ischemic insult via HDAC inhibition.
METHODS:
To investigate the protective effects of C. nutans against stroke. Primary cortical neurons were subjected to oxygen/glucose deprivation-reoxygenation (H-R) *in vitro* hypoxic model. MTT assay was used to detect cell viability. Flowcytometry was used to monitor mitochondrial membrane potential (MMP), apoptosis. Reporter assay was used to detect the transcriptional activity of HDAC1/6. RT/PCR and Western blot were used to detect HDACs mRNA and protein expression. For *in vivo* study, rats were subjected to 3-vessel occlusion (MCAO)/reperfusion. Neurological deficit scores and infarct volumes were used to evaluate functional and cellular damage.
RESULTS:
In vitro: An early induction of HDAC1/2/3/6/8 was noted in neurons after OGD insult. C. nutans extract selectively inhibited HDAC1 and HDAC6 expression, increased cell viability, maintained MMP and attenuated neuronal apoptosis compared to vehicle control at hypoxia 30mins and 1 day reperfusion (H0.5R24). Moreover, C. nutans suppressed hypoxia-induced HDAC1 and HDAC6 transcription. Besides ameliorating neuronal death, C. nutans also protected astrocytes and endothelial cells from hypoxic-induced cell death.
In vivo: C. nutans significantly reduced infarct brain volumes in rat subjected to 30-min ischemia and 1-day reperfusion. Furthermore, C. nutans improved motor functional recovery even after 2-weeks reperfusion.

CONCLUSIONS:
In the present study we reported that C. nutans suppressed post-hypoxic HDACs activation and neuronal death and confers therapeutic potential for ischemic brain damage. This study further opens a new avenue for the use of herbal medicines to via epigenetic regulation control brain injury.

Disclosures: J. wu: None. **H. Tsai:** None. **M. Kao:** None. **W. Cheung:** None. **T. Lin:** None.

Poster

729. Neurovascular and Metabolic Coupling: Normal Brain and Stroke

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 729.25/BBB1

Topic: C.08.Stroke

Title: Multiple presentations with plausible CADASIL diagnosis

Authors: *F. P. MELENDEZ[1], D. CASADESUS[2], E. MALA[1], K. SHILLINGFORD[1], C. A. VILLANIA[1], W. ZELEZNAK[1];
[1]Ross Univ. Sch. of Med., Miramar, FL; [2]Jackson Hlth. Syst., Miami, FL

Abstract: Introduction: CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy) has variable presentations that mirror other neurological disorders. We offer 3 cases with different presentations in which CADASIL was a plausible diagnosis.
Case Description:
Case 1: 50yo African American woman with PMH of multiple sclerosis (MS) diagnosed 20 years ago, who complained of diplopia. She had been treated by multiple physicians for MS without improvement. She had a prior admission for acute hemorrhage to the right medial midbrain and pons and was discharged home with minimal sequelae. MRI identified multiple areas of hyperattenuation within the white matter which led to the suspicion of CADASIL. Genetic testing confirmed mutation in the NOTCH3 gene and she was treated symptomatically.
Case 2: 48yo Caucasian male with PMH of alcohol dependence, depressive disorder and neurosyphilis was admitted for inappropriate behavior, AMS, ataxia, seizure and unexplained fall. His Hx of alcohol abuse led to the suspicion of Wernicke's encephalopathy which was treated with thiamine. CTA of the neck showed malformation of vertebral arteries and MRA demonstrated fetal-type circulation. Family members were contacted and confirmed a positive family Hx of CADASIL.
Case 3: 43yo Hispanic male with PMH of HTN, obesity, Hx of multiple suicide attempts and polysubstance abuse was admitted for AMS and right sided weakness. He was hypertensive at

246/156mmHg with an NIHSS of 10. Brain CT showed multiple hypodensities within the left caudate nucleus, subcortical and deep white matter related to vessel ischemic changes; confirmed by MRI and MRA. Cerebral angiogram revealed bilateral segmental stenosis in the distal MCA, ACA and PCA branches consistent with vasculitis. Repeat MRI 15 days later, showed new punctate infarcts to the left cerebellum and right corona radiata. NOTCH3 genetic testing was unavailable, therefore, due to no prior family Hx of CADASIL, it was concluded that the etiology of multiple ischemic strokes was likely due to cerebral vasoconstriction and hypertensive urgency. Patient was treated with anti-hypertensive medication and discharged home. At outpatient follow-up 2 weeks later, MRI showed an acute infarct of the ventral pons and patient was subsequently readmitted.
Discussion: CADASIL is a rare disease that has many manifestations. These cases demonstrate a few symptoms, which along with clinical evaluation and detailed history can guide the physician towards an accurate diagnosis. Nonetheless, mutation in the NOTCH3 gene and a positive family Hx of CADASIL are alone, confirmatory for the disease.

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Poster

729. Neurovascular and Metabolic Coupling: Normal Brain and Stroke

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 729.26/BBB2

Topic: C.08.Stroke

Title: Heparan sulfate targets at $VEGF_{165}$ bioactivity enhances vascular angiogenesis: Potential vascular therapy for stroke.

Authors: *S. CHAN[1,2], K. HAYAKAWA[1], A. SMITH[2], V. NURCOMBE[2], E. LO[1];
[1]Radiology, Massachusetts Gen. Hosp., Charlestown, MA; [2]Inst. of Med. Biology, Agency for Science, Technol. and Res. (A*STAR), Singapore, Singapore

Abstract: Brain angiogenesis has been found to exert positive effect in long term recovery among ischemic stroke patients. Several attempts using proangiogenic factor, for instance $VEGF_{165}$ in preclinical stroke experiments is by far encouraging. Despite being one of the most active proangiongenic factor, high concentration of $VEGF_{165}$ was reported to induce vascular permeability. Several studies showed that heparan sulfate proteoglycan glycosaminoglycan (HSPG) plays important roles in modulating VEGF bioactivity. However the specific mechanism remains unresolved. Recent study indicated that HSPC provides high order control of VEGF receptors activation. Here, we have successfully isolated the heparan sulfate glycoprotein

(HS7+ve) that binds to and enhances the bioactivity of $VEGF_{165}$, selective to VEGF receptor 2 (VEGFR) pathway. Our results indicated that HS7+ve complexed with $VEGF_{165}$, significantly increased RBE4 cells proliferation. Furthermore, HS7+ve, when complexed with $VEGF_{165}$, enhanced RBE4 cells tube formation and cell migration. The pro-angiogenic potential of HS7+ve was further confirmed by the activation of VEGFR2 pathway in which, HS7+ve activates pVEGFR2, pErk1/2 and pAkt. To access the HS7+ve effects on vascular permeability, RBE4 cells were treated with oxygen glucose deprivation (OGD) and IL-1β, followed by the detection of the adherence and tight junction proteins. Finally the pro-angiogentic potential of HS7+ve was further assessed in an *in vivo* hemorrhagic model. Our current results demonstrate the potential beneficial effect of glycotherpeutic agents targeting at the augmentation of $VEGF_{165}$ bioactivity against stroke.

Disclosures: **S. Chan:** A. Employment/Salary (full or part-time): Research fellow (full time). **K. Hayakawa:** A. Employment/Salary (full or part-time): Assistant Professor (full time). **A. Smith:** A. Employment/Salary (full or part-time): Research fellow (full time). **V. Nurcombe:** A. Employment/Salary (full or part-time): Principal Investigator (full time). **E. Lo:** A. Employment/Salary (full or part-time): Professor (full time).

Poster

729. Neurovascular and Metabolic Coupling: Normal Brain and Stroke

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

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Topic: C.09. Brain Injury and Trauma

Support: National Eye Institute Grant 1 R01 EY024481

Title: Voltage-gated potassium channels are implicated in zinc-mediated retinal ganglion cell death following optic nerve injury

Authors: *R. M. SAND[1], Y. LI[2], L. I. BENOWITZ[2], P. A. ROSENBERG[3];
[1]Neurobio., [2]Neurosurg., [3]Neurol., Boston Children's Hosp., Boston, MA

Abstract: Unlike neurons in the peripheral nervous system, those in the brain and spinal cord are unable to regenerate damaged axons. As a result, injury or disease often leads to permanent motor and cognitive deficits. To address this problem, we have investigated the signaling pathways that promote cell survival and axon regeneration in the central nervous system (CNS) using the mouse retina and optic nerve as a model. We have recently shown that after a crush injury in the optic nerve, free zinc (Zn^{2+}) levels rise significantly in retinal ganglion cells before dying (see poster by Li et al., this meeting). When Zn^{2+} is chelated at the time of crush, cell

survival rates are approximately twice those of control retinas. Remarkably, and unlike most other interventions reported to date, this survival benefit is long lasting, and observed as late as 12 weeks after injury. Since voltage-gated potassium channel (Kv) activation is known to underlie cell shrinkage during apoptotic cell death, we hypothesized that Kv might be involved in the zinc-mediated degeneration of retinal ganglion cells. We tested this hypothesis by injecting several different Kv inhibitors, alone or in combination with the zinc chelator ZX1, into the eye at the time of optic nerve crush. Agents were injected again four days later, and retinal ganglion cell survival and axon regeneration were quantified two weeks post-crush. We found that injecting a low dose of tetraethylammonium (TEA) mimicked and occluded the neuroprotective effect of zinc chelation, confirming that TEA-sensitive Kv share the zinc pathway with respect to cell survival. However, the same dose of TEA blocked the increase in regeneration caused by zinc chelation. At this dose (100 μM injected into the vitreous), TEA only targets members of the Kv1, Kv3, and Kv7 family. Therefore, we tested more specific peptide and small molecule inhibitors of these subfamilies to tease apart the contributions of the various channels to retinal ganglion cell survival and axon regeneration. We also looked at the amount and pattern of expression of specific channel types using immunohistochemistry with and without optic nerve crush. Ultimately, this work will help to illuminate the molecular mechanisms of zinc-mediated neurodegeneration in the retina and in the CNS and may provide new therapeutic avenues for treating neurodegenerative diseases of the eye and brain as well as traumatic CNS injury.

Disclosures: **R.M. Sand:** None. **Y. Li:** None. **L.I. Benowitz:** None. **P.A. Rosenberg:** None.

Poster

729. Neurovascular and Metabolic Coupling: Normal Brain and Stroke

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Topic: C.08.Stroke

Support: NIH grant R01 NS061953

Title: A large fraction of glutamate release in the ischemic penumbra is mediated by redox-dependent changes in membrane permeability

Authors: *A. A. MONGIN[1], P. DOHARE[3], N. H. BOWENS[1], A. VIPANI[1], V. R. YADAV[2], Y.-X. WANG[2], P. J. FEUSTEL[1], R. W. KELLER, Jr.[1];
[1]Dept. of Neurosci. and Exptl. Therapeut., [2]Dept. of Mol. and Cell. Physiol., Albany Med. Col., Albany, NY; [3]Dept. of Pediatrics, New York Med. Col., Valhalla, NY

Abstract: In animal models of stroke, antioxidants potently protect against ischemic brain damage. However, for unclear reasons, the antioxidant treatments have shown little to no clinical benefits in human stroke. While exploring the basis for antioxidant efficacy, we recently found that the superoxide dismutase (SOD) mimetic tempol strongly reduced intraischemic release of the excitotoxic neurotransmitter glutamate (Glu), and this effect correlated with histological and behavioral neuroprotection [P. Dohare et al. FRBM 77: 168, 2014]. Antioxidants with other mechanisms of action did not modify pathological excitotoxin levels, suggesting a new Glu-dependent mechanism for protection by SOD mimetics. Here, we explored the pathways that mediate the redox-sensitive Glu release in rodent transient focal ischemia. Pre-, intra-, and postischemic Glu levels were measured in the rat cortex, in ischemic penumbra using a microdialysis approach combined with 2-h occlusion of the middle cerebral artery. Several candidate release mechanisms were explored by delivering via microdialysis perfusate the following pharmacological inhibitors: dihydrokainate (to block the glial Glu transporter GLT-1), DCPIB (volume-regulated anion channels), 18α-glycyrrhetinic acid (connexin hemichannels), probenecid (pannexins), and L-serine-O-sulfate (cystine/glutamate antiporter). Among the tested inhibitors, only DCPIB and dihydrokainate produced modest (~20-30%) reductions in pathological Glu levels, prompting a question about what pathway mediates the remaining large fraction of the excitotoxin release. To model and explore potential mechanism(s), we exposed primary cultures of glial cells to (a) metabolic inhibition, (b) oxidative stress, or (c) their combination. Only the latter condition produced sustained and non-selective changes in the membrane permeability for amino acids and larger molecules. Such changes were completely dependent on elevations in the intracellular [Ca^{2+}] and likely mediated by activation of phospholipases. Overall, our results paint an unexpectedly complex picture of multimodal redox-sensitive Glu release and tissue damage in clinically relevant ischemic penumbra. The improved understanding of stroke pathology will help in developing new treatment modalities.

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Poster

730. Effects of Diet on Brain And Behavior

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Program#/Poster#: 730.01/BBB5

Topic: G.08. Drugs of Abuse and Addiction

Support: 1R21DA03873801A1

5R03DA03828702

Title: Cytoplasmic FMR1-interacting protein 2 (Cyfip2) is a major genetic factor underlying binge eating

Authors: *C. D. BRYANT[1], S. L. KIRKPATRICK[1], L. R. GOLDBERG[1], N. YAZDANI[1], K. I. LANDAVERDE[1], R. K. BABBS[1], J. WU[1], D. F. JENKINS[2], E. R. REED[3], A. BOLGIONI[1], K. P. LUTTIK[1], V. KUMAR[4], W. E. JOHNSON[2], M. K. MULLIGAN[5], P. COTTONE[1]; [1]Pharmacol. and Exptl. Therapeut., [2]Computat. Biomedicine, [3]Bioinformatics, Boston Univ. Sch. of Med., Boston, MA; [4]The Jackson Lab., Bar Harbor, ME; [5]Genetics, Genomics and Informatics, Univ. of Tennessee Hlth. Sci. Ctr., Memphis, TN

Abstract: Eating disorders are lethal and heritable; however the genetic factors are unknown. Binge eating is a highly heritable quantitative trait associated with eating disorders, obesity and neuropsychiatric dysfunction. Identifying the genetic basis of binge eating is essential to understanding neurobiological mechanisms and the development of pharmacotherapeutics. We developed a binge eating paradigm and assessed genetic differences in C57BL/6 substrains that are nearly genetically identical. Quantitative trait locus (QTL) mapping in an F_2 cross followed by gene knockout was used to identify and validate the causal genetic factor. Transcriptome analysis of the striatum via mRNA sequencing (RNA-seq) was used to reveal potential neurobiological mechanisms that bridge genetic variation with behavior. Outbred CFW mice and C57BL/6NJ (B6NJ) inbred mice showed a robust escalation in palatable food (PF) consumption and conditioned food reward whereas the closely related C57BL/6J substrain (B6J) did not. We identified a single genome-wide significant QTL on chromosome 11 that mapped precisely to the location of a missense mutation in *Cyfip2* (cytoplasmic FMRP-interacting protein, 2) that was recently identified for cocaine-induced neurobehavioral plasticity. We validated *Cyfip2* as a causal genetic factor using mice heterozygous for a deletion in *Cyfip2* that demonstrated a complete reversal of compulsive-like binge eating. Transcriptome analysis of the striatum via RNA-seq from a subset of F_2 and knockout mice identified an enrichment of genes associated with psychiatric disorders (eating, substance abuse, depression, bipolar, and anxiety) that likely underlie the neurobiological mechanisms. We also identified differentially spliced genes such as *Homer1*, *Arpp21*, *Map4*, and *Gls* (glutaminase) whose isoforms could functionally contribute to binge-induced structural neuroplasticity in the striatum. To summarize, to our knowledge, *Cyfip2* is the first genome-wide significant genetic factor to be identified for binge eating in mammalian model organisms or humans and demonstrates the utility of our model and approach for future gene mapping efforts in populations with increased genetic complexity.

Disclosures: **C.D. Bryant:** None. **S.L. Kirkpatrick:** None. **L.R. Goldberg:** None. **N. Yazdani:** None. **K.I. Landaverde:** None. **R.K. Babbs:** None. **J. Wu:** None. **D.F. Jenkins:** None. **E.R. Reed:** None. **A. Bolgioni:** None. **K.P. Luttik:** None. **V. Kumar:** None. **W.E. Johnson:** None. **M.K. Mulligan:** None. **P. Cottone:** None.

Poster

730. Effects of Diet on Brain And Behavior

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 730.02/BBB6

Topic: G.03. Emotion

Support: NARSAD N018940

Title: Effects of diet and obesity on anxiety-like behaviors in obesity-prone and obesity-resistant rats

Authors: *P. J. VOLLBRECHT[1], Y. ALONSO-CARABALLO[2], A. M. CHADDERDON[2], A. L. MATTHEWS[2], E. M. JUTKIEWICZ[2], C. R. FERRARIO[2];
[1]Biol., Hope Col., Holland, MI; [2]Univ. of Michigan, Ann Arbor, MI

Abstract: Epidemiological data suggest that body mass index and obesity are strong risk factors for depression and anxiety. However, it remains difficult to separate cause from effect, as predisposition for obesity may enhance susceptibility to anxiety, or vice versa. Here, we examined the effects of diet and obesity on anxiety-like behaviors in selectively-bred obesity-prone and obesity-resistant rats. This model enables us to distinguish effects of pre-existing differences vs. weight gain on anxiety-like behaviors. Food intake and weight gain was recorded over 4 weeks. Measures were made in male rats given standard lab chow (Lab Diet 5001) or 60% high-fat diet (Open Source Diets D12492). Elevated plus maze and open field testing were used to evaluate anxiety-like behaviors, and forced swim and sucrose preference testing were used to examine pro-depressive like behaviors. Prior to weight gain, behavior in the elevated plus maze was similar between obesity-prone and obesity-resistant groups. However, after spontaneous increases in adiposity and weight in chow fed obesity-prone rats, anxiety-like behaviors in the elevated plus maze and open field tests were enhanced in obesity-prone vs obesity-resistant rats. Furthermore, the magnitude of these anxiety-like behaviors was positively correlated with weight gain in obesity-prone, but not obesity-resistant rats. No differences in forced swim behavior were found between groups. Importantly, locomotor behavior in the home cage and standard locomotor chambers was similar between groups, suggesting that differences in anxiety-like behaviors only emerge in anxiogenic environments. Consumption of a 60% high-fat diet produced substantial weight gain in obesity-prone rats, and weight gain in obesity-resistant rats that was comparable to that of obesity-prone rats fed standard chow. However, high-fat diet induced obesity was not sufficient to enhance anxiety-like behaviors in obesity-resistant rats. No further increases in anxiety-like behavior were found in obesity-prone rats, likely due to floor effects. Together, these data suggest that enhanced anxiety-like behavior in obesity-prone rats is not due to basal differences, but instead emerges with the development of obesity.

Disclosures: **P.J. Vollbrecht:** None. **Y. Alonso-Caraballo:** None. **A.M. Chadderdon:** None. **A.L. Matthews:** None. **E.M. Jutkiewicz:** None. **C.R. Ferrario:** None.

Poster

730. Effects of Diet on Brain And Behavior

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Program#/Poster#: 730.03/BBB7

Topic: F.02. Behavioral Neuroendocrinology

Support: NIEHS P01 ES002848 Project 3

USEPA 83543401 Project 3

Title: Effects of perinatal bisphenol A and high fat diet on adult anxiety and social behavior

Authors: *L. M. WISE, S. M. BOAS, J. M. JURASKA;
Dept. of Psychology, Univ. of Illinois, Champaign, IL

Abstract: Bisphenol A (BPA) is an environmental endocrine disruptor that has been detected in the vast majority of the United States population. The current study explores the combined effects of relevant doses of BPA and diet on anxiety and social behaviors. Pregnant dams consumed doses of 0, 40, or 400 ug/kg/day of BPA from gestational day 2 through parturition. After birth, the pups individually consumed the same dose of BPA from postnatal day 1 through 10. The pregnant dams were also fed control or high-fat diet from gestational day 0 (day sperm plug detected) through postnatal day 10.
Elevated Plus Maze. Anxiety behavior in adulthood was assessed via the elevated plus maze. The animals were allowed one five-minute trial to explore an elevated plus maze. Preliminary results suggest a significant sex difference in the number of entries onto the open arms of the maze. However, there was no effect of diet or BPA dose the number of entries onto the open or closed arms of the maze. Also, there was no effect of diet or BPA dose on the time spent in the open or closed arms of the maze.
Social Recognition. Social memory in adulthood was assessed via the social recognition paradigm. A same sex juvenile at was placed in the home cage of the experimental animal, after which the juvenile was removed for a delay period of 15, 45, 90 or 120 minutes. Following the delay, a familiar and novel juvenile were introduced into the adult cage and the amount of time the adult animal spent investigating each juvenile was recorded. There was a significant sex difference in the amount of time spent investigating the familiar and the novel juveniles. However, there was no effect of BPA or high-fat diet exposure on social memory.

Disclosures: **L.M. Wise:** None. **S.M. Boas:** None. **J.M. Juraska:** None.

Poster

730. Effects of Diet on Brain And Behavior

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Program#/Poster#: 730.04/BBB8

Topic: F.02. Behavioral Neuroendocrinology

Support: P01 ES002848-Project 3

USEPA 83543401-Project 3

T32 ES007326

Title: Long-term behavioral effects of perinatal exposure to phthalates and maternal high-fat diet in male and female rats

Authors: *J. WILLING[1], D. G. KOUGIAS[2], L. R. CORTES[1], C. M. DRZEWIECKI[2], K. E. WEHRHEIM[1], J. M. JURASKA, 61820[1];
[1]Psychology, [2]Neurosci. Program, Univ. of Illinois, Champaign, IL

Abstract: Endocrine disruptors and high-fat diets pose major health concerns in our industrialized society. Phthalates, a class of endocrine disrupting chemicals commonly used as plasticizers or solvents, can possess anti-androgenic, estrogenic, and anti-estrogenic activities, as well as suppress other steroid enzymes. Humans are ubiquitously exposed to many different phthalates through a variety of consumer goods, with diet presumed to be the main source of exposure for some phthalates. Moreover, phthalates are more prevalent in fatty foods. Both phthalates and high-fat diets can increase oxidative stress and inflammation, suggesting the need to examine the potential for interactive effects between them. In humans, the gestational period appears to be a particularly vulnerable window to phthalates' endocrine and metabolic disrupting activity. Since the prenatal period through postnatal day (P)10 in rats approximately corresponds to prenatal cortical development in humans, in the present study, we use a rat model of human prenatal exposure to investigate the long-term cognitive effects of gestational/neonatal exposure to phthalates and a high-fat diet. Dams were fed cookies containing an environmentally relevant mixture of phthalates at doses of 0, 200, or 1000 µg/kg/day from the second day of gestation until P10. They also consumed high-fat or control diets during this period. When the pups of treated animals reached adulthood (~P90), behavior was assessed in the elevated plus maze, attentional set shift, acoustic startle, prepulse inhibition, open field, and novel object recognition tasks. Preliminary data indicates that perinatal exposure to high fat diets result in increased

prepulse inhibition. Preliminary data also suggests effects of perinatal phthalate exposure and interactions with the sex of the animal or high fat diet on all measures except the elevated plus maze. Results from these studies will provide insight into the sex-specific effects of developmental exposure to ubiquitous endocrine disruptors and maternal diet on cognitive and emotional behavior.

Disclosures: J. Willing: None. **D.G. Kougias:** None. **L.R. Cortes:** None. **C.M. Drzewiecki:** None. **K.E. Wehrheim:** None. **J.M. Juraska:** None.

Poster

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Program#/Poster#: 730.05/BBB9

Topic: F.02. Behavioral Neuroendocrinology

Support: P01 ES002848-Project3

USEPA 83543401 Project 3

Title: Perinatal exposure to phthalates and a high-fat diet affects maternal behavior, indices of pup development, and periadolescent behavior

Authors: *D. G. KOUGIAS[1], L. R. CORTES[2], S. G. RHOADS[2], J. M. JURASKA[3]; [1]Neurosci. Program, [2]Psychology Dept., [3]Neurosci. Program & Psychology Dept., Univ. of Illinois at Urbana-Champaign, Champaign, IL

Abstract: Endocrine disruptors and high-fat diets pose major health concerns in our industrialized society. Humans are ubiquitously exposed to phthalates, a class of endocrine disrupting chemicals commonly used as plasticizers or solvents, through a variety of consumer goods and food. Phthalates can possess anti-androgenic, estrogenic, and anti-estrogenic activities, as well as suppress the synthesis of steroidogenic enzymes. They are also more prevalent in fatty foods, which are readily available in the developing world and likely contribute to the increase in obesity rates. Since phthalates and high-fat diets are both capable of increasing oxidative stress and inflammation, which are known to negatively affect cerebral function and plasticity, it is important to study phthalates and high-fat diets together. In humans, the gestational period appears to be a particularly vulnerable window to phthalates' endocrine and metabolic disrupting effects. In this study, we use a rat model of human prenatal exposure to investigate the developmental and behavioral effects of phthalates and a high-fat diet. Since the prenatal period through postnatal day (P)10 in rats approximately corresponds to prenatal cortical

development in humans, the dams were orally dosed (0, 200, or 1000 μg/kg/day) with a mixture of phthalates throughout this period. The dams were also fed high-fat or control diets during this period. Total caloric intake, initial litter size and sex ratio, dam and pup bodyweights, and pup anogenital distance were recorded during the dosing period. Preliminary data indicates that pups had higher body weights at P10 and P25 from litters with dams fed a high-fat diet, and effects of phthalate exposure on body weight varied with age. Also, maternal behavior was observed daily throughout the neonatal period (P3-P15), and dams fed a high-fat diet licked their offspring more and engaged in more nest reorganization. During the periadolescent period, social play behavior will be observed and pubertal onset determined.

Disclosures: D.G. Kougias: None. **L.R. Cortes:** None. **S.G. Rhoads:** None. **J.M. Juraska:** None.

Poster

730. Effects of Diet on Brain And Behavior

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Program#/Poster#: 730.06/BBB10

Topic: F.02. Behavioral Neuroendocrinology

Support: NIA - 1K01AG044466 to PLJ

NIH R01 MH 052619 to AS

Title: Effects of high fat diet on body composition, food and caloric intake, behavioral and thermoregulatory responses of ovariectomized female Wistar rats following estrogen replacement

Authors: *A. R. ABREU[1,2], I. F. CALIMAN[7,3], C. S. BERNABE[3,4,5], R. C. A. DE MENEZES[1], A. SHEKHAR[2,4,6], P. L. JOHNSON[3,4];
[1]Dept. of Biol. Sci., UFOP, Ouro Preto, Brazil; [2]Psychiatry, [3]Anat. and Cell Biol., [4]Stark Neurosciences Res. Inst., [5]Program in Med. Neurosciences, [6]Indiana Clin. and Translational Sci. Inst., Indiana Univ. Sch. of Med., Indianapolis, IN; [7]Physiological Sci., Federal Univ. of Espirito Santo, Vitoria, Brazil

Abstract: Background - Hot flash associated vasomotor symptoms (VMS), which emerge during perimenopause occur in ~70% of women and can persist for years, are thought to contribute to sleep and mood disruption. Another common symptoms that emerges during menopause is weight gain, and recent large cohort clinical studies have determined that increased adiposity is associated with more problematic VMS during menopause transition and early postmenopause

stages (*Thurston et al., 2013 Fert Steril)* and weight loss produces less VMS (*Thurston et al., 2014 Menopause*). Methods - Using an ovariectomy rat model of a surgical perimenopausal state +/- estrogen replacement (ER), we investigated the effects of a 9 week high fat diet (HFD), compared to normal diet (ND), on weight, body mass composition (assessed with an EchoMRI), food and caloric intake, mood/anxiety behaviors and thermoregulatory activity. Results - Following 9 weeks, ND controls and HFD rats has similar increases in weight gain, but even though HFD rats consumed less food it represented more calories and produced higher adiposity. ER was equally effective in reducing weight gain and adiposity in ND and HFD rats. VMS-associated activity was assessed by measuring tail skin temperatures (TST). Although there were no differences in TSTs at baseline or following a VMS inducing drug (systemic injection of FG-7142, an anxiogenic GABA disenhancing drug) in either HFD or ND rats, ER reduced TST activity at baseline which produced a diminished TST responses post FG-7142. Behavioral assessments of anxiety/mood disruption revealed that avoidance behaviors were exacerbated in HFD rats which were reduced with ER. There were no acoustic startle response differences noted between the HFD and ND rats, but ER did reduce startle responses in ND rats. Using a novel object short term memory recognition test, only ER treated HFD and ND OVEX rats showed evidence of recognizing of a novel object (no diet effects were noted). Conclusions - These results showed that although a HFD does not produce worse weight gain post OVEX it was associated with higher fat composition, which among all other measures was only noted to increase avoidance behaviors. ER was effective in reducing weight gain, fat composition, and VMS-associated TST activity, as well as reducing evidence of mood/anxiety disruption (increases in avoidance and startle responses) and restoring object recognition.

Disclosures: **A.R. Abreu:** None. **I.F. Caliman:** A. Employment/Salary (full or part-time): Indiana University School of Medicine. **C.S. Bernabe:** None. **R.C.A. De menezes:** None. **A. Shekhar:** None. **P.L. Johnson:** None.

Poster

730. Effects of Diet on Brain And Behavior

Location: Halls B-H

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Program#/Poster#: 730.07/BBB11

Topic: F.04. Stress and the Brain

Title: Modulation of acute stress-induced anorexia by fatty acid amide hydrolase inhibition in rats

Authors: *M. A. STICHT[1], K. A. SHARKEY[1], M. N. HILL[2];
[1]Dept. of Physiol. and Pharmacol., [2]Dept. of Cell Biol. and Anat. and Psychiatry, Univ. of Calgary, Calgary, AB, Canada

Abstract: In rats, acute stress is known to cause a reduction in food intake. This anorectic effect is likely modulated by factors such as stressor intensity, as well as motivation to feed, i.e. hunger/satiety. Many aspects of the stress response are regulated by the endocannabinoid system (ECS), as is food intake, but it is unclear how this system modifies stress effects on feeding. Given that fatty acid amide hydrolase (FAAH) inhibition has previously been shown to attenuate a variety of stress effects through an increase in the endocannabinoid, anandamide (AEA), we tested the hypothesis that AEA regulates stress-induced anorexia. As such, male Sprague-Dawley rats were administered vehicle or the FAAH inhibitor PF04457845 (PF) systemically or via intracerebroventricular (icv) cannulation prior to an acute psychological stressor (restraint stress). Post-stress feeding intake of regular lab diet (Prolab RMH 2500) and animal body weight were subsequently assessed over the following 24hr and 3 days, respectively.
Stress-induced anorexia was not observed when rats were fasted for 24hr prior to a 2hr stressor or with a shorter stress period of 1hr. Among non-food-deprived animals, following exposure to a 2hr restraint stressor (or a 2hr fast to control for food intake in home cage controls), stressed animals consumed significantly less chow within the first hour of testing (1.9g vs 5.4g), and feeding was significantly reduced up to 22 hr post-stress. However, the anorectic effects were not altered by systemic (10 mg/kg) or icv (30 μg) PF administration. Interestingly, PF was found to reduce food intake and normal body weight gain in non-stressed control animals compared to vehicle-treated rats. Taken together, these results suggest that stress-induced effects on feeding are influenced by satiety/hunger signals, which may be affected similarly by FAAH inhibition alone. Current experiments are exploring the mechanism(s) of action underlying this paradoxical effect of FAAH inhibition on stress-induced feeding behaviour.

Disclosures: **M.A. Sticht:** None. **K.A. Sharkey:** None. **M.N. Hill:** Other; Hill is a consultant for Pfizer.

Poster

730. Effects of Diet on Brain And Behavior

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 730.08/BBB12

Topic: G.08. Drugs of Abuse and Addiction

Support: NSERC

CRC

Title: Should we turn off the lights? The modulation of light-cue value in the augmentation of heroin seeking in chronically food restricted rats

Authors: *F. SEDKI[1], L. MAYERS[2], D. RIZZO[2], S. TRIEU CHAO[2], J. COHEN[2], T. D'CUNHA[2], U. SHALEV[2];
[1]CSBN, [2]Psychology, Concordia Univ., Montreal, QC, Canada

Abstract: Disruptions in energy balance can affect motivated behaviors. For example, caloric restriction can increase drug taking and seeking in rats. Recently, we reported an augmented heroin seeking in food restricted rats under withdrawal. The underlying motivational mechanisms driving this effect are unclear. It is possible that exposure to caloric restriction may enhance the incentive value attributed to the drug-associated cues, and in turn augment drug seeking. It is important however to distinguish the incentive value of a light cue independent of the value acquired through its association with a drug. Thus, we investigated the effect of food restriction on the acquisition of a new operant response reinforced solely by heroin- or non-heroin-associated cues. In a separate experiment, we compared heroin- with non-heroin-associated light seeking in a discrete choice paradigm. Male Long-Evans rats were trained lever-press for heroin, for 10 days. Next, rats were moved to the animal colony and maintained on free access to food (Sated) or subjected to 14 days of mild chronic food restriction (FDR). On day 14, rats were tested in the self-administration chambers. Ex.1: During self-administration training, heroin infusions were associated with light/tone cues. On test day, the incentive value of the cues was assessed by training the rats on a novel nose-poke response reinforced solely by the heroin-associated cues. Ex.2: Responses on one lever led to a heroin infusion, and activation of a light/tone cue. Responses on a second lever activated a different light/tone or just a tone cue. On test day, rats were treated as described in Ex.1. Ex.3: Rats were trained on a discrete choice trial, with one lever associated with heroin infusions+light cue, and another lever associated only with a different light cue. On test day rats were exposed to choice trials reinforced by cues alone. Ex.1 results: Rats in the FDR group acquired the novel behavior at a greater rate compared to the Sated group. Ex.2: FDR-induced increase in nose-poke response rate was observed for both heroin-paired and the alternative light cue. Ex.3: Responses for a heroin-associated light cue was greater than responses on for the light cue alone in both Sated and FDR groups. Response rate for the light alone however was greater in the FDR versus Sated group. Our findings suggest that food restriction increases the incentive salience of environmental stimuli, including, but not exclusively, of drug-paired cues.

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Poster

730. Effects of Diet on Brain And Behavior

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Topic: G.08. Drugs of Abuse and Addiction

Support: Funded by the NSERC Discovery Program

Title: The role of the paraventricular nucleus of the thalamus in the augmentation of heroin seeking by chronic food restriction

Authors: *A. CHISHOLM, J. IANNUZZI, U. SHALEV;
Psychology, Concordia Univ., Montreal, QC, Canada

Abstract: Drug addiction is a chronic disorder that is characterized by compulsive drug seeking and involves switching between periods of abstinence and relapse. In both human and animal models of addiction chronic food restriction has been shown to increase rates of relapse. Previously, our laboratory has demonstrated a robust increase in drug seeking following a period of withdrawal in chronically food-restricted rats compared to sated rats. To date, the neural mechanisms that mediate the effect of chronic food restriction on drug seeking have not been elucidated. However, the paraventricular nucleus of the thalamus (PVT) appears to be a promising target to investigate. The PVT is uniquely placed to contribute to both homeostatic control and drug seeking systems. Thus, the objective of the current study was to study the effect of PVT inactivation on heroin seeking under food restriction conditions.
Prior to heroin self-administration training, male Long Evans rats were injected with a viral vector carrying an inhibitor Designer Receptor Exclusively Activated by Designer Drug (DREADD) into the PVT. Next, rats were trained to self-administer heroin over 10 days (0.1 mg/kg/infusion; i.v.). Following training, rats were removed from the operant conditioning chambers and were placed into drug withdrawal for 16 days. Over the withdrawal period, rats were exposed to a mild food restriction (90% of baseline body weight) or were given unrestricted access to food. On the 14th and 16th day of the withdrawal period, two drug-seeking tests were conducted in which rats were injected (i.p.) with either CNO (3 mg/kg), to activate the DREADDs, or vehicle, 20 minutes prior to test. Injectors' placement was verified using immunohistochemistry.
All rats reliably learned to self-administer heroin. As expected, food-restricted rats demonstrated an augmented heroin seeking during the heroin-seeking test in comparison to sated rats. PVT inactivation seemed to reduced heroin seeking in all rats regardless of the feeding condition, however this effect did not reach statistical significance.
These results suggest that PVT activity appears to play a role in heroin seeking. However, PVT activity may not modulate the augmentation of heroin seeking following chronic food restriction.

Disclosures: **A. Chisholm:** None. **J. Iannuzzi:** None. **U. Shalev:** None.

Poster

730. Effects of Diet on Brain And Behavior

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Program#/Poster#: 730.10/BBB14

Topic: G.08. Drugs of Abuse and Addiction

Support: NSERC

FRSQ

CRC

Title: The role of glutamate neurotransmission in the augmentation of heroin seeking induced by chronic food restriction

Authors: ***T. M. D'CUNHA**, M. RUSSO, S. LE NOBLE, A. USYPCHUK, J. IANNUZZI, F. SEDKI, U. SHALEV;
Psychology, Concordia Univ., Montreal, QC, Canada

Abstract: Caloric restriction increases the risk of relapse during drug abstinence in human drug addicts. Our laboratory has demonstrated that chronic food restriction augments heroin seeking following prolonged withdrawal in rats with a history of heroin self-administration. During the heroin-seeking test, food restricted (FDR) rats had elevated extracellular levels of dopamine (DA) in the nucleus accumbens (NAc), and administration of a DA D1 receptor antagonist into the nucleus accumbens decreased heroin seeking in the FDR rats. The NAc receives a multitude of glutamatergic innervation from a variety of brain regions, and previous work has demonstrated that glutamate transmission in the NAc can interact with dopaminergic activity. In this study we investigated extracellular glutamate levels in the NAc shell and core, as well as the role of AMPA receptors in the augmentation of heroin seeking induced by chronic food restriction. First, rats were trained to self-administer heroin for 10 days. Next, rats were moved to the animal colony for 14 days of drug withdrawal. During the withdrawal period some rats were given unrestricted access to food and others were subjected to a mild chronic food restriction until they reached approximately 90% of their original body weight. On the 14^{th} day of food restriction rats were returned to the operant conditioning chambers for a heroin-seeking test under extinction conditions. Extracellular glutamate was assessed using *in vivo* microdialysis and HPLC during baseline conditions in the animal facility and then during the 3 h heroin-seeking test. Food restriction significantly augmented heroin seeking compared to the sated rats. There

were no significant changes in extracellular glutamate levels between the FDR and sated rats. Bilateral administration of the AMPA receptor antagonist NBQX (0.0, 0.3, 1.0 µg/side) into the NAc core decreased heroin seeking but only in the sated rats. Taken together, these results suggest that glutamate transmission in the NAc through AMPA receptors is not involved in the augmentation of heroin seeking induced by chronic food restriction. Future studies will investigate if NMDA or metabotropic glutamate receptors play a role in food restricted-induced augmentation heroin seeking.

Disclosures: **T.M. D'Cunha:** None. **M. Russo:** None. **S. le Noble:** None. **A. Usypchuk:** None. **J. Iannuzzi:** None. **F. Sedki:** None. **U. Shalev:** None.

Poster

730. Effects of Diet on Brain And Behavior

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Topic: G.08. Drugs of Abuse and Addiction

Support: NSERC Discovery Grant (US)

Title: Changes in the AMPA/NMDA ratio in neurons of the nucleus accumbens shell associated with chronic food restriction and with withdrawal from heroin self-administration

Authors: ***U. SHALEV**, I. GLOVACI, T. D'CUNHA, A. CHAPMAN;
Concordia Univ., Montreal, QC, Canada

Abstract: Chronic food restriction increases drug craving and the risk to relapse during abstinence in human addicts. Recently, we demonstrated that chronic food restriction augments heroin seeking during withdrawal in rats with a history of heroin self-administration. These food-restricted (FDR) rats demonstrated changes in dopamine transmission in the nucleus accumbens (NAc). Previous research has shown that dopamine in the NAc can modulate glutamatergic synaptic transmission, however, this has not been studied in the framework of food restriction and heroin seeking. Therefore, the purpose of the current study was to assess synaptic plasticity induced by heroin self-administration and by chronic food restriction during heroin withdrawal in the NAc shell. Changes in AMPA and NMDA receptor mediated currents were evaluated in both sated and FDR Long-Evans rats under withdrawal from heroin. Briefly, half of the rats tested were trained to self-administer heroin for 10 days in operant conditioning chambers. Then, rats were moved to the animal colony for 14 days of withdrawal during which one group was food restricted to approximately 90% of their original body weight (Heroin-FDR), or given unrestricted access to food (Heroin-Sated). The second half of the animals were not heroin

trained and served as controls. Of these, one group was free-fed (Naïve-Sated) and the other group was food restricted (Naïve-FDR). In vitro whole cell patch-clamp recordings were performed on the 14th day of withdrawal for all animals. Voltage-clamp recordings were obtained from visually-identified NAc shell medium spiny neurons. AMPA currents were measured at -70 mV, as well as at +40 mV before and after application of the NMDA antagonist APV, and NMDA currents were quantified by subtraction. Cells recorded in the Naïve-FDR group demonstrated a marked reduction in NMDA, but not AMPA, currents, resulting in a strongly increased AMPA/NMDA ratio relative to cells in the Naïve-Sated group. Additionally, our initial results also suggest a decrease in the AMPA/NMDA ratio following heroin administration and withdrawal in the Heroin-Sated group, due to an increase in NMDA currents. In contrast, both AMPA and NMDA currents in the Heroin-FDR group were similar to those in the Naïve-Sated group. Taken together, our results suggest differential effects of heroin self-administration and chronic food restriction during withdrawal on glutamatergic synapses in the nucleus accumbens shell.

Disclosures: **U. Shalev:** None. **I. Glovaci:** None. **T. D'Cunha:** None. **A. Chapman:** None.

Poster

730. Effects of Diet on Brain And Behavior

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Topic: F.04. Stress and the Brain

Support: DARPA W911NF1010093

Title: Individual differences in the microbiome of rats resilient or vulnerable to the effects of chronic social defeat stress

Authors: *J. PEARSON-LEARY[1], K. BITTINGER, 19128[3], C. TANES, 19128[2], S. BHATNAGAR[1];
[2]CHOP Microbiome Ctr., [1]Children's Hosp. of Philadelphia, Philadelphia, PA; [3]Dept. of Microbiology, Perelman Sch. of Medicine, Univ. of Pennsylvania, Philadelphia, PA

Abstract: Chronic exposure to stress is associated with increased incidence of depression, generalized anxiety and PTSD. However, stress induces vulnerability to such disorders only in a sub-population of individuals, as others remain resilient. To model these individual differences in response to stress, our lab, and others, have shown that two subpopulations of Sprague-Dawley rats emerge with repeated social defeat. Approximately half of defeated rats exhibit short latencies (SL) to be defeated averaged over 7 days whereas the other half of rats resist defeat and

exhibit increased latency. SL rats have increased anxiety-like and depressive-like behaviors suggesting that they are stress vulnerable. Behaviorally, LL rats are no different from controls, suggesting they are stress resilient. A current hypothesis is that inflammation mediates vulnerability to chronic stress. Previous data has shown that inflammation can be mediated by the gut microbiome. We hypothesized that vulnerability to stress would be characterized by an inflammation-promoting gut microbiome. We performed shotgun metagenome sequencing on fecal pellets of male Sprague Dawley rats collected prior to, and after social defeat, and nondefeated control rats. SL and LL rats were identified by cluster analyses on latencies to social defeat. In the pre-stress samples, there were no significant differences between control, SL, or LL rats, suggesting that pre-existing differences in the gut microbiome do not predict vulnerability or resiliency. However, repeated social defeat produced robust changes in microbiome composition at the community level. In the LL and SL groups, we found that *Lactobacillus*, *Blautia*, and *Eubacterium* were among the top increased taxa, and social defeat produced a significant increase in the ratio of firmicutes:bacteriodetes. *Collinsella* increased dramatically in LL rats. In the SL rats, four of the five taxa identified were members of the class Clostridia. Clostridia are especially important for regulating immune function. Because SL rats exhibit indices of increased inflammation, we examined this taxon and observed that Clostridia were indeed significantly increased in SL rats. Thus, analyses of gut microbiome profiles suggests that vulnerability and resiliency have unique effects on the microbiome, with vulnerability associated with increased clostridia, which may promote the inflammatory phenotype previously observed in SL/vulnerable rats. Ongoing studies are determining the physiological and neural consequences of increased clostridia in SL rats and the mechanisms by which such effects might be mediated.

Disclosures: **J. Pearson-Leary:** None. **K. Bittinger:** None. **C. Tanes:** None. **S. Bhatnagar:** None.

Poster

730. Effects of Diet on Brain And Behavior

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 730.13/BBB17

Topic: F.04. Stress and the Brain

Support: Danone Nutricia Research

Title: Caesarean delivery induces enduring behavioural and physiological effects in mice: reversal by selective targeting of the microbiota-gut-brain axis

Authors: *L. H. MORAIS[1], A. MOYA-PEREZ[2], A. VENTURA-SILVA[2], G. CLARKE[2], K. REA[2], I. RENES[5], J. KNOL[5], S. WANG[6], C. STANTON[3], T. DINAN[4], J. F. CRYAN[2]; [2]APC Microbiome Inst., [3]Teagasc Food Res. Centre, Moorepark, [4]Psychiatry, [1]Univ. Col. Cork, Cork, Ireland; [5]Danone Nutricia Res., Utrecht, Netherlands; [6]Danone Nutricia Res., Singapore, Singapore

Abstract: Increasing evidence points to a role for the microbiome in regulating many aspects of health including brain health. It is now clear that the microbiota-gut-brain axis plays a key role in regulating behaviour and brain function. Early-life is a critical developmental window for the microbiome and brain. The initial seeding of the gut microbiome occurs during birth as the infant emerges through the mother's birth canal. However, birth by Caesarean section (C-section) results in a different pattern of bacterial colonisation compared to naturally delivered offspring. Recently, we have demonstrated that C-section delivered mice present with enduring behavioural and physiological phenotypes. The aim of the present study was to investigate whether these effects were reversible by oral supplementation with prebiotic, probiotic or synbiotics (combination of prebiotics and probiotics) that target the microbiota-gut-brain axis. We assessed a number of translational behavioural and physiological outputs in early-life and in adulthood that are relevant to neuropsychiatric disorders. Following birth by C-Section or *per vaginum* pups were exposed to a dietary intervention with specific probiotic, prebiotic or synbiotic mixtures given to dams during lactation period. Male offspring was weaned to their mother's diet at postnatal day 21 till 14 weeks at which they were sacrificed. Probiotic treatment was administered in the drinking water and the prebiotic was incorporated into the custom-made rodent diet. In early-life, all dietary interventions (prebiotics, probiotics and synbiotics) reversed C-section-mediated increase in ultrasonic vocalisation and impairment in maternal attachment at postnatal day 9 and 10. In adulthood at week 8, the three interventions restored C-section mediated deficits in social cognition and novel object recognition memory. Prebiotic and probiotic treatment independently reduced anxiety in C-section born offspring exposed to the marble burying test, while the probiotic itself displayed anxiolytic effect in the natural born offspring. Furthermore, synbiotics also reduced depression-like behaviour in the natural born. Ongoing analysis at the level of the gastrointestinal tract, the immune system and the brain will illuminate mechanistic insights underlying such effects. In conclusion, our data highlight the importance of the gut microbiome in early life in shaping neurodevelopment and stress-related behaviours. Further, it demonstrated that there are potential microbiota-based strategies to counteract such effects.

Disclosures: L.H. Morais: None. **A. Moya-Perez:** None. **A. Ventura-Silva:** None. **G. Clarke:** None. **K. Rea:** None. **I. Renes:** A. Employment/Salary (full or part-time): Danone Nutricia Research. **J. Knol:** A. Employment/Salary (full or part-time): Danone Nutricia Research. **S. Wang:** A. Employment/Salary (full or part-time): Danone Nutricia Research. **C. Stanton:** None. **T. Dinan:** None. **J.F. Cryan:** None.

Poster

730. Effects of Diet on Brain And Behavior

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Program#/Poster#: 730.14/BBB18

Topic: G.03. Emotion

Support: Colorado College Research and Development Grant

Colorado College Faculty/Student Collaborative Grant

Title: Gender differences in adolescent probiotic influence on anxiety

Authors: *L. L. DRISCOLL[1], T. TUMMINO[2], W. HARRIS[2], S. COOKE[2], R. LACH[2]; [1]Colorado Col., Colorado Spgs, CO; [2]Colorado Col., Colorado Springs, CO

Abstract: Nervous system function and psychological health are influenced by the unique composition of one's gut microbiome. However, little is known about the influence of microbiome composition on brain function later in life, and the extent to which gut microbes exert their influence on development of anxious or depressive behaviors through direct neural communication with the brain via the vagus nerve. The purpose of the current study was to elucidate the effects of oral supplementation of the probiotic strain *Bifidobacterium infantis* 35624 on the development of anxiety and depression in healthy adolescent rats, and to identify the unique contribution of the vagus nerve to such effects. As weanlings, rats were subjected to either a subdiaphragmatic vagotomy or a sham surgery and supplemented daily with *B. infantis* or vehicle for two weeks. Anxious behavior in the elevated plus maze (EPM), and depressive behavior in the forced swim test (FST), were assessed in young adulthood. *B. infantis* treatment decreased anxious behavior in a vagally-dependent manner for male rats and in a vagally-independent manner for female rats. In contrast, neither probiotic supplementation nor vagotomy altered FST immobility time. These results suggest that the vagus nerve plays a necessary role in in the anxiolytic effects of *B. infantis* in male rats, but that hormonal or immune benefits conferred by *B. infantis* are sufficient to mediate anxiolysis in female rats.

Disclosures: L.L. Driscoll: None. **T. Tummino:** None. **W. Harris:** None. **S. Cooke:** None. **R. Lach:** None.

Poster

730. Effects of Diet on Brain And Behavior

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Program#/Poster#: 730.15/BBB19

Topic: F.04. Stress and the Brain

Support: R21 DA038504

Title: Anorexia increases sensitivity to cocaine-induced locomotion: evidence for dysregulation of dopamine transporters

Authors: *C. M. GEORGE[1], W. A. OWENS[1], C. P. FRANCE[2], G. M. TONEY[1], L. C. DAWS[3];
[1]Physiol., [2]Pharmacol. and Psychiatry, [3]Physiol. and Pharmacol., Univ. Hlth. Sci. Ctr. At San Antonio, San Antonio, TX

Abstract: Anorexia Nervosa (i.e., anorexia) is a major public health concern compounded by a lack of effective treatments. This complex disorder is most common in females and often manifests during adolescence. Although the etiology of anorexia remains unclear, dysregulation of dopamine (DA) neurotransmission is a consistent finding. The DA transporter (DAT) terminates DA neurotransmission by high-affinity uptake of DA into neurons and is therefore a primary regulator of the strength and duration of DA signaling. Given the important role that DAT plays to clear dopamine together with strong evidence of dysregulated DA neurotransmission in anorexia-related eating disorders, it is possible that DAT function is aberrant in individuals with anorexia. Surprisingly, however, few studies have investigated DAT activity in anorexia. Toward this end, we used the "activity-based anorexia" (ABA) model in rats, which recapitulates key characteristics of anorexia, including hyperactivity and restricted food intake. As an index of DAT function *in vivo*, we examined the effect of the DAT blocker cocaine on locomotion. Adolescent rats with one hour access to food ate less than those with ad libitum access to food, regardless of whether they had access to locked or unlocked running wheel. We determined that "anorexic" adolescent female rats were more sensitive to cocaine than female control rats as evidenced by a leftward shift of the ascending and descending limbs of their dose-response curves. Moreover, females that underwent ABA were more sensitive to the effects of cocaine on locomotion than their male counterparts. Preliminary data generated using high-speed chronoamperometry to measure transporter function *in vivo* indicate that DAT activity was increased in females, but not males, exposed to ABA. Together, these data support the hypothesis that during adolescence, DAT function in females is susceptible to reduced food intake, to hyperactivity or to both in combination, which might contribute to development or severity of anorexia-related behavior.

Disclosures: **C.M. George:** None. **W.A. Owens:** None. **C.P. France:** None. **G.M. Toney:** None. **L.C. Daws:** None.

Poster

730. Effects of Diet on Brain And Behavior

Location: Halls B-H

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Program#/Poster#: 730.16/BBB20

Topic: F.04. Stress and the Brain

Support: R01 MH093320-01

R01 MH106978

Title: Antidepressant-like effects of ketamine in serotonin transporter mutant mice and organic cation transporter 3 knockout mice

Authors: *M. A. BOWMAN[1], W. KOEK[2], L. C. DAWS[3];
[2]Psychiatry and Pharmacol., [3]Physiol. and Pharmacol., [1]Univ. of Texas Hlth. Sci. Ctr. San Anto, San Antonio, TX

Abstract: Twenty percent of adults are diagnosed with major depressive disorder, which is typically treated with selective serotonin reuptake inhibitors (SSRIs). However, this type of medication takes approximately six weeks to produce therapeutic effects. Recently, low doses of ketamine, a noncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist, have been shown to produce rapid and long-lasting antidepressant effects. Studies that have begun to examine the mechanisms underlying these effects have focused on intracellular changes mediated by NMDA and/or AMPA receptors. However, the traditional view of the etiology of depression involves the need for an increase in extracellular serotonin to regulate mood. Surprisingly, there appears to be little research into the effects of ketamine on serotonin. One study found an increase in extracellular serotonin while another found an inhibition of serotonin uptake following ketamine treatment. Together, these studies suggest a role for serotonin in the antidepressant-like effects of ketamine, and putatively one involving the serotonin transporter (SERT). To further elucidate this role, we examined antidepressant-like effects of ketamine in SERT mutant mice bred on a C57BL/6J background. SERT knockout, heterozygous, and wildtype adult mice were treated with vehicle or 30 mg/kg ketamine and tested either 24 hours or 7 days later in the tail suspension test, a validated model for detecting antidepressant drug effects. Ketamine had antidepressant-like effects only in SERT knockout mice. Taken together with published findings that SERT knockout mice have increased expression of organic cation transporter 3 (OCT3) relative to wildtype littermates, and that blockade of OCT3 produces

antidepressant-like effects and inhibits serotonin uptake, these results raise the possibility that OCT3 plays a role in antidepressant-like effects of ketamine. Ongoing studies are investigating the dose-response relationship of these effects in SERT mutant mice and in OCT3 knockout mice to investigate a potential role for OCT3 in antidepressant-like effects of ketamine.

Disclosures: M.A. Bowman: None. **W. Koek:** None. **L.C. Daws:** None.

Poster

731. Monoamines, Amino Acids, and Other Regulators of Energy Balance

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 731.01/BBB21

Topic: F.10. Food Intake and Energy Balance

Support: University of San Diego Office of the Provost

Title: Dopamine modulation of sleep and feeding in *Drosophila*

Authors: *A. PAVIN, M. DRISCOLL, D. SITARAMAN;
Dpt. of Psychological Sci., Univ. of San Diego, San Diego, CA

Abstract: Neuromodulators such as dopamine (DA) and serotonin have long been implicated in innate and learned behaviors. Widespread dopaminergic system manipulations depleting DA levels result in sleep regulation deficits in *Drosophila Melanogaster* (the fruit fly). Further evidence suggests that regulation of feeding is also adversely affected when DA levels are diminished. The fly brain contains a total of 200 dopaminergic neurons, 130 of which innervate the mushroom body (MB). The MB is an associative learning network important for decision-making as well as for the control of sleep in *Drosophila.* Approximately 2,000 kenyon cells (KCs) make up the lobes of the MB and synapse onto MB output neurons (MBON) in 15 non-overlapping compartments. The 130 DANs project into these compartments, modulating the synaptic strength of these connections. Activation of specific DANs has been demonstrated to significantly reduce sleep in *Drosophila*. It remains unclear whether the observed sleep deficits are a cause of dopaminergic regulation of arousal, or of a motivational behavior like feeding. Using *Drosophila* as an experimental system, a comprehensive screen of all MB DANs and MBONs was conducted. We analyzed specific subsets of DAN and ON clusters in order to elucidate dopamine's role in the regulation of feeding and motivational behaviors. The goal of these data is to explore a link between dopamine regulation of motivational behaviors and their implications for sleep regulation.

Disclosures: A. Pavin: None. **M. Driscoll:** None. **D. Sitaraman:** None.

Poster

731. Monoamines, Amino Acids, and Other Regulators of Energy Balance

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 731.02/BBB22

Topic: F.10. Food Intake and Energy Balance

Title: Feeding regulates sphingolipid-mediated signaling in mouse hypothalamus.

Authors: *V. VOZELLA[1], N. REALINI[1], A. MISTO[1], D. PIOMELLI[1,2];
[1]D3, Fondazione Inst. Italiano Di Tecnologia, Genova, Italy; [2]Dept. of Anat. and Neurobio., Univ. of California, Irvine, CA

Abstract: BACKGROUND:
Feeding behavior and energy metabolism are regulated by a complex network of central and peripheral signals, of which the hypothalamus is an especially important node (Schwartz MW et al., 2000). The sphingolipids - a class of lipid-derived messengers that include ceramide (Cer), dihydroceramide (dHcer), sphingosine (SO) and sphingosine-1-phosphate (SO1P) - have been recently implicated in the pathogenesis of metabolic dysfunction (Russo SB et al., 2013; Bikman BT, 2012), but the physiological signals regulating their formation and deactivation in the hypothalamus are unknown.
OBJECTIVES:
In the present study, we investigated the effect of food deprivation or high-fat diet (HFD) on sphingolipid levels and on the expression of enzymes involved in sphingolipid metabolism in the hypothalamus.
METHODS:
Male C57BL/6J mice (n=5/group) were subjected to four different feeding conditions: 1) free feeding (FF); 2) 12h food deprivation (FD); 3) 1h refeeding after FD; 4) 6h refeeding after FD. To study the effect of HFD, C57BL/6J mice were divided into two groups: standard diet (2.66 kcal/g) and HFD (5.24 kcal/g) and killed at different time points (1-3-7-14-28 days). Hypothalamic sphingolipids were extracted, identified and quantified by liquid chromatography/mass spectrometry. Gene expression of enzymes involved in sphingolipid biosynthesis was evaluated by qPCR.
RESULTS:
Relative to free feeding, fasting: 1) decreased SO1P levels; 2) increased SO, the precursor of SO1P; 3) reduced the levels of dihydroceramide (d18:0/18:0), a product of *de novo* pathway of ceramide biosynthesis; 4) down-regulated transcription of sphingosine kinases (SK) and ceramide synthase. After 1 day and 14 days, free feeding mice exposed to a HFD showed lower levels of Cer24:0, its precursor dHCer 24:0, and Cer24:1 compared to mice fed standard diet. Significant decreases in SO1P were also observed after 7 and 14 days of HFD.
CONCLUSION:

Intracerebroventricular injections of SO1P reduce food intake and stimulate energy expenditure in rats (Silva VRR et al., 2014). Our results suggest that hypothalamic levels of SO1P, its precursor SO and enzymes involved in the conversion of one into the other (SK) are influenced by the feeding status. Feeding also regulates other aspects of sphingolipid metabolism, suggesting additional roles for this lipid pathway in the control of energy balance.

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Poster

731. Monoamines, Amino Acids, and Other Regulators of Energy Balance

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 731.03/BBB23

Topic: F.10. Food Intake and Energy Balance

Support: NPTC-PRODEP-15

Title: Effect of resveratrol on food intake, lipid oxidation, GABA levels in rats hypercaloric diets fed

Authors: ***C. SANDOVAL SALAZAR**[1], L. MOSQUEDA-VERA[2], V. BELTRÁN-CAMPOS[2], M. SOLÍS-ORTIZ[3], J. RAMIREZ-EMILIANO[3];
[1]Univ. De Guanajuato, Celaya, Mexico; [2]Enfermería y Obstetricia, Univ. de Guanajuato, Celaya, Mexico; [3]Ciencias Médicas, Univ. de Guanajuato, Leon, Mexico

Abstract: Obesity is associated with psychological factors, genetic predisposition and food habits. Diet composition influence total energy intake and long-term changes to body weight and body composition. Some studies reported that chronic intake of a hypercaloric diet produce changes in the oxidative stress which accompanied by accelerate lipid peroxidation. Natural products, such as flavonoids found in fruits like resveratrol have potential therapeutic agents recognized for their antioxidant activity. The aim of this study was to explore the effect of antioxidant resveratrol, lipids damage oxidative in the frontal cortex of rats hypercaloric diets fed. A total of 50 healthy male rats were divided equally into six groups: first group: the first group= hypercaloric diet 1 (HPC1: sugar 5% + lard 15%), the second group= HPC1 + resveratrol, the third group= (HPC2: sugar 10% + lard 10%), the fourth group= HPC2 + resveratrol, the fifth group= (HPC3: sugar 15% + lard 5%) and the sixth group= HPC3 + resveratrol, the seventh and eighth groups were fed with standard diet (SD) and SD + resveratrol. All the groups were fed during five months, however in the fifth month the second, fourth, sixth and eighth groups received resveratrol during four weeks. Food intake was recordly daily. Lipid peroxidation levels were analyzed by measuring thiobarbituric acid reactive substances. GABA

levels were determinate with ELISA kit. The SD-fed rat intake was approximately 7.77% more lower than the HPC groups and the resveratrol have no effect on food intake. The lipid peroxidation were high in HPC2 and HPC3. The resveratrol decreases lipid peroxidation in the HPC3 group with significant differences. The GABA levels in the HPC-fed rats demonstrated a significant decrease compared with the SD group in the frontal cortex. The GABA levels in the HPC3 + resveratrol appeared to be more elevated without reaching to SD group. This results indicate that exposure to hypercaloric diet decrease the GABA concentration and increase de lipid oxidation in the frontal cortex; this probably suggesting a imbalance in inhibitory process of frontal cortex.

Disclosures: C. Sandoval Salazar: None. **L. Mosqueda-vera:** None. **V. Beltrán-campos:** None. **M. Solís-ortiz:** None. **J. Ramirez-emiliano:** None.

Poster

731. Monoamines, Amino Acids, and Other Regulators of Energy Balance

Location: Halls B-H

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Topic: F.10. Food Intake and Energy Balance

Support: he Council for Science, Technology and Innovation, SIP, "Technologies for creating next-generation agriculture, forestry and fisheries"

Title: Chrono-nutrition effect of theanine intake on the learning memory and anxiety in mice.

Authors: *A. MUTO, T. SAKAI, Y. TAHARA, Y. KIKUCHI, T. SHIRAISHI, M. YAMASAKI, H. MOTOHASHI, S. SHIBATA;
Fac. of Sci. and Engin., Waseda Univ., Tokyo, Japan

Abstract: Theanine is an amino acid included in green tea, is known to interact with glutamate receptors in the brain nervous system. In the previous studies, theanine has improved the learning memory deficit in mice, and also anxiety in patients with schizophrenia. Circadian rhythm system is important to control the morning /evening difference of physiological functions such as not only body temperature, blood pressure and endocrine secretion, but learning/memory and anxiety. Circadian rhythm system also controls the timing effect of drug intake (chrono-pharmacology) and food/nutrition intake (chrono-nutrition). Therefore, the timing of drug/food may affect their effects. Although human usually takes green tea during daytime but not during late nighttime, there has been no study that has evaluated the effect of theanine on learning memory and anxiety in the morning or evening. Male ICR 3-week-old mice were allowed to acclimatize themselves to the chow (AIN-93G) and tap water for 1 week under a 12-h/12-h

light/dark schedule. After then, the mice were given tap water or 0.3% theanine included tap water ad libitum for 3 weeks. Mice were tested by novel object recognition test for learning/memory and open field test for anxiety. Maintain of learning memory was seen in theanine intake group when novel objective recognition test was conducted in the morning or evening, and theanine was more effective in the morning group compared with evening group. Anti-anxiety effect of theanine was appeared in the morning, but not in the evening evaluated by open field test. In conclusion, theanine was effective for learning memory maintenance and also anti-anxiety with observation of stronger effect in the morning test than the evening test. In the future experiment, we will investigate effect of consistent timing of both theanine intake and behavioral test. This work was partially supported by the Council for Science, Technology and Innovation, SIP, “Technologies for creating next-generation agriculture, forestry and fisheries” (funding agency: Biooriented Technology Research Advancement Institution, NARO)

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Poster

731. Monoamines, Amino Acids, and Other Regulators of Energy Balance

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Program#/Poster#: 731.05/BBB25

Topic: F.10. Food Intake and Energy Balance

Support: DGAPA IN 224214

CONACYT Posdoctoral fellowship for Dr. Cruz-Trujillo

Title: The dopamine D4 receptor antagonist L-745,870 suppresses food intake and food-reinforced behavior

Authors: R. CRUZ-TRUJILLO[1], L. UBERLA-JIMENEZ[1], J. MANCILLA-DIAZ[1], V. LOPEZ ALONSO[1], A. GUTIÉRREZ-HERNÁNDEZ[2], *R. ESCARTIN-PEREZ[1];
[1]UNAM, FES Iztacala, Tlalnepantla de Baz, Mexico; [2]Inst. Politécnico Nacional, Ciudad de México, Mexico

Abstract: Due to the high prevalence of obesity in many countries, the study of eating and food-reinforced behavior has become a priority. Several studies have shown that dopamine and its receptors are involved in normal and pathological regulation of food intake. For example, it has been reported that the selective activation of D4 dopamine receptors (D4R) in the paraventricular nucleus of the hypothalamus (PVN) produced a stimulatory effect on standard food intake by

increasing the release of glutamate. Accordingly, it has been suggested that alterations in the hypothalamic dopaminergic signaling pathways that regulate energy homeostasis may facilitate the development of obesity-associated caloric overeating by a D4R dependent mechanism. Accordingly, the aim of this study was to evaluate the effects of the central administration of D4 receptor antagonist, L-745,870, on food intake (standard lab chow) and on the motivation for palatable food (sucrose pellets). Adult male Wistar and Sprague Dawley rats were stereotaxically implanted with microinjection cannulas in the PVN and in the Nucleus Accumbens Shell to locally administrate L-745,870 (1 ug/0.5 uL) and consumption of standard food (1 and 2 hours after injection, Wistar rats) and the motivation for palatable food (45 mg pellets, operant test procedure, Sprague Dawley rats) were evaluated. We found that injection of L-745,870 (1 ug/0.5 uL) in the PVN significantly suppressed standard food intake in the first hour after the intra-hypothalamic injection. Furthermore, local administration of L-745,870 (1 ug/0.5 uL) in the Nucleus Accumbens Shell significantly decreased food-reinforced lever pressing on a progressive ration reinforcement schedule (PR1000). Our results showed that the central D4R modulate feeding behavior by inhibiting motivational and hedonic processes, suggesting that this compound may be useful for the pharmacological treatment of obesity.

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Poster

731. Monoamines, Amino Acids, and Other Regulators of Energy Balance

Location: Halls B-H

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Program#/Poster#: 731.06/BBB26

Topic: F.10. Food Intake and Energy Balance

Support: NIH Grant DA025634

Title: Electrical stimulation of the ventral noradrenergic bundle results in frequency dependent norepinephrine release in the paraventricular nucleus of the hypothalamus

Authors: *A. I. GERTH, M. F. ROITMAN;
Psychology, Univ. of Illinois At Chicago Dept of Psych, Chicago, IL

Abstract: The paraventricular nucleus of the hypothalamus (PVN) plays a critical role in a variety of regulatory and homeostatic functions including the autonomic response to stress as well as feeding behavior. Norepinephrine (NE) inputs to the PVN arise from the nucleus of the solitary tract (NTS) and project via the ventral noradrenergic bundle (VNB) to the the parvocellular, dorsomedial portion of the PVN. NTS NE neurons are robustly activated by the

satiety-inducing feeding peptide cholecystokinin and the malaise- inducing agent lithium chloride - supporting a role in intake suppression. They also appear to be critical for the robust food consumption induced by central delivery of agents that interfere with glucose sensing. Thus, the nature of the NE PVN input remains somewhat murky and sampling NE in the PVN will help to resolve its role. Here, we sought to characterize NE release in the PVN using fast-scan cyclic voltammetry - an electrochemical technique that can sample the release of electroactive neurotransmitters, like NE with good analyte specificity and excellent temporal and spatial resolution. In anesthetized rats, a carbon fiber microelectrode was lowered into the dorsomedial parvocellular region of the PVN and its voltage was modulated (-0.4V to +1.3V and back [scan], 400V/s, 10scans/s). Once every 5 minutes, the VNB was stimulated (60pulses, 60Hz, 200μA). A reliable increase in current was observed. Representative cyclic voltammagrams from each experiment were regressed against those obtained during post-recording calibration in a flow injection system where the electrode was exposed separately to 1uM NE and 1uM epinephrine (E). Cyclic voltammograms obtained in vivo compared favorably to NE but not E ($r^2 = 0.84 \pm 0.02$ vs 0.53 ± 0.02, respectively). In vivo NE release was frequency dependent. No release was detected for stimulation frequencies under 10Hz but higher frequencies led to greater release (75.3 ± 6.4 vs 248 ± 35.3 vs 283.45 ± 3.8 nM for 10, 30 and 60 Hz, respectively). Measurement and characterization of phasic NE release in the PVN will aid in resolving its role in feeding behavior.

Disclosures: **A.I. Gerth:** None. **M.F. Roitman:** None.

Poster

731. Monoamines, Amino Acids, and Other Regulators of Energy Balance

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Topic: F.10. Food Intake and Energy Balance

Support: NIH Grant R01NS079584

Title: A dopaminergic circuitry critical for homeostatic protein intake in *Drosophila*

Authors: *L. CHIU, Q. LIU, S. LIU, L. KODAMA, W. HORIUCHI, J. DANIELS, D. BALDONI, M. N. WU;
Johns Hopkins Univ., Baltimore, MD

Abstract: The regulation of food intake is essential for an organism's survival and fitness. During this process, living organisms need to select between food sources that vary in quality and quantity. While progress has been made in our understanding of neural regulation of energy

intake, much less is known about how specific nutrients, such as protein intake is regulated. Accumulating evidences suggest that protein consumption is homeostatically regulated and may have a profound effect on total energy intake. Here we identified a small subset of dopamine (DA) neurons specifically involved in homeostatic regulation of protein intake in *Drosophila.* In order to assess the consumption of protein-containing food, we utilized two behavioral assays in our study. Blue-dye feeding assay was applied to quantify the absolute amount of protein intake and the two-choice assay was performed to measure animals' preference to protein relative to sugar. Female flies have previously been shown to undergo a post-mating dietary switch, with increased intake and preference for protein-enriched food. From a circuit-based screen, we found that DA signaling is required for this post-mating dietary switch. In addition, virgin female and male flies that have been protein-deprived exhibited a similar dietary switch, which was also suppressed by silencing DA signaling. Using an intersectional approach, we identified two DA neurons from the PPM2 subgroup (DA-WED) that are required for increasing consumption and preference of protein-containing food following protein deprivation. Moreover, activation of these neurons is sufficient to induce a protein preference in protein-satiated animals. Functional imaging experiments reveal that the activity of these DA-WED neurons correlates with the physiological drive for protein intake. Together, our study reveals a critical circuit mechanism by which *Drosophila* adjust their dietary strategy to maintain protein homeostasis.

Disclosures: L. Chiu: None. **Q. Liu:** None. **S. Liu:** None. **L. Kodama:** None. **W. Horiuchi:** None. **J. Daniels:** None. **D. Baldoni:** None. **M.N. Wu:** None.

Poster

731. Monoamines, Amino Acids, and Other Regulators of Energy Balance

Location: Halls B-H

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Boettcher young investigator award

Narsad young investigator award

Title: Characterizing motivational and dopaminergic changes resulting from distinct behavioral histories of sucrose access

Authors: *N. ELTOM, K. J. PULTORAK, D. GOMEZ, E. OLESON;
Psychology, CU Denver, Aurora, CO

Abstract: Approximately 2/3 of adults in the United States are either overweight or obese. Studies have shown that on average, Americans consume about 500-800 more calories per day than needed because of the availability of food, more specifically, carbohydrate rich and sucrose enriched foods (Flegal et al, 2000). Characterizing the effects of sucrose access on motivational and neurochemical markers of addiction may help to elucidate the neural basis of obesity. In the present study we are providing rats with either no access, intermittent access or unlimited access to sucrose in their home operant boxes in 23hr cycles over 28 days. Over the 28-day history, we assess for overall and circadian changes in: food intake, sucrose intake, water intake and weight gain. We then use a novel behavioral economic food seeking task and the progressive ratio schedule to assess for changes in motivation for sucrose. Motivation is assessed twice: once immediately after the 1-month history, and then again following a period in which all groups were deprived of sucrose. Immediately after the first motivational assessment, we use fast-scan cyclic voltammetry to assess for changes in transient dopamine responses to sucrose and a conditioned predictor of sucrose access.

Disclosures: **N. Eltom:** None. **K.J. Pultorak:** None. **D. Gomez:** None. **E. Oleson:** None.

Poster

731. Monoamines, Amino Acids, and Other Regulators of Energy Balance

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Topic: F.10. Food Intake and Energy Balance

Support: DVAMC VISN 10 Research Initiation Program

LSC VA Medical Research and Education Foundation

Title: Aromatic amino acid metabolites generated by the intestinal microbiome are present in human cerebrospinal fluid

Authors: *G. E. JASKIW[1], M. E. OBRENOVICH[2], Z. RENLIANG[4], C. J. DONSKEY[3];
[2]Res. Service, [3]Infectious Dis. Service, [1]Louis Stokes Cleveland DVAMC, Cleveland, OH;
[4]Proteomics and Metabolomics Core, Lerner Res. Inst. at Cleveland Clin., Cleveland, OH

Abstract: Elevated urinary levels of the aromatic amino acid metabolite 3-(3-Hydroxyphenyl)-3-hydroxypropionic acid (HPHPA) have been reported in patients with schizophrenia and autism (Shaw, 2010). While HPHPA is thought to be generated by the intestinal microbiome and

derived from either tyrosine (TYR) (Curtius et al, 1976), or phenylalanine (PHE) (Shaw, 2010), the precise origin and distribution of HPHPA is not well-defined. We used liquid chromatography/mass spectroscopy (LC/MS) to measure HPHPA/ isomer and other aromatic amino acid metabolites in 3 experiments: 1) *Clostridium difficile*, a pathogen that colonizes the intestinal tract, was cultured *in vitro* on standard medium and the resulting levels of HPHPA and other metabolites were determined; 2) L-TYR or L-PHE 400mg/kg IP was administered to adult mice (Harlan, CFI, female) and metabolites were measured in urine harvested 2h later; 3) Human CSF samples from our clinical laboratory were analyzed. We found that *C. difficile* generated HPHPA/ isomer as well as 3-hydroxyphenyl-propionic acid (HPPA) (another intermediary in aromatic amino acid metabolism) and the structurally similar compound 3,4-Dihydroxyhydrocinnamic acid (DHCA). In mice, the administration of L-TYR, but not L-PHE, resulted in elevated urinary HPHPA / isomer. In human CSF, HPHPA/ isomer and DHCA were detected. Thus, we confirm earlier findings that components of the intestinal microbiome can generate aromatic amino acid metabolites. In mice, we demonstrated that urinary HPHPA/ isomer is derived at least in part from L-TYR. Furthermore, at least some of the analytes generated by the intestinal microbiota can cross the human blood brain barrier. This raises the possibility that aromatic amino acid metabolites differentially generated by gut bacteria in hosts with neuropsychiatric disorders, could directly affect brain function. Further studies will delineate the synthesis, distribution and neuroactive properties of these compounds.

Disclosures: **G.E. Jaskiw:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); shares of ITCI. F. Consulting Fees (e.g., advisory boards); Guidepoint Global. **M.E. Obrenovich:** None. **Z. Renliang:** None. **C.J. Donskey:** None.

Poster

731. Monoamines, Amino Acids, and Other Regulators of Energy Balance

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Topic: F.10. Food Intake and Energy Balance

Support: 2014R1A6A1029617

2014R1A1A1037655

2014R1A2A1A11051231

Title: Peptide hormone A has a role in the hypothalamic control of appetite

Authors: *Y. JANG[1,2], J. HAN[1,2], S. KIM[1,2], J. KIM[1,2], M. LEE[1,2], I. RYU[1,2], M. RYU[1,2,3], J. HEO[1,2,4], G. KWEON[1,2,3];
[1]Dept. of biochemistry, [2]Dept. of Med. Sci., [3]Res. Inst. for Med. Sci., [4]Brain research institute, Chungnam Natl. Univ. Sch. of Med., Daejeon, Korea, Republic of

Abstract: Hypothalamic regulation of appetite governs whole body energy balance. Inability of satiety induction is etiologic implication of obesity. Despite the pharmaceutical agents are used to modulate this alteration of anorectic signaling, unexpected effects on neurological or cardiac system are not the issue to disregard. The current study announced that an endogenous peptide hormone A (PHA) had an anorectic effect in hypothalamus. We demonstrated that proopiomelanocortin (POMC) and agouti-related protein (AgRP) neurons, which are the targets of anorectic leptin hormone, located in arcuate nucleus (ARC) of hypothalamus co-expressed PHA. In addition, intracerebroventricular injection of PHA peptide significantly suppressed food intake in mice via increasing α-melanocotye-stimulating hormone (α-MSH) content in hypothalamus. We addressed that the anorectic effect of PHA is presumably via induction of phosphorylation of ERK and reduction of AMPK phosphorylation. Notably, PHA provoked the expression of Janus kinase 2 (JAK2) phosphatases known to be involved in the feedback inhibition of leptin signaling, whereas PHA expression was promoted by STAT3 phosphorylation induced by leptin receptor activation. We suggest that PHA is abundantly expressed in hypothalamus to evoke the anorectic melanocortin pathway and mediates leptin signaling (2014R1A6A1029617, 2014R1A1A1037655, 2014R1A2A1A11051231).

Disclosures: **Y. Jang:** None. **J. Han:** None. **S. Kim:** None. **J. Kim:** None. **M. Lee:** None. **I. Ryu:** None. **M. Ryu:** None. **J. Heo:** None. **G. Kweon:** None.

Poster

731. Monoamines, Amino Acids, and Other Regulators of Energy Balance

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Topic: F.10. Food Intake and Energy Balance

Support: Human Frontier Science Program

Medical Research Council

Title: The diverse roles and organisation of lateral hypothalamic inhibitory neurons

Authors: *C. KOSSE[1,2], D. BURDAKOV[1,3];
[1]The Francis Crick Inst., London, United Kingdom; [2]Univ. Col. London, London, United Kingdom; [3]MRC Ctr. for Developmental Neurobio., King's Col., London, United Kingdom

Abstract: The lateral hypothalamus is well known for its role in body weight control, which traditionally has been assigned to weight-loss promoting orexin and weight-gain promoting MCH neurons. Recently, different LH GABAergic neurons that are distinct from MCH and orexin neurons have been described to effect body weight as well. These neurons might be the missing piece to elucidate the LH circuitry underlying body weight control and explaining how weight gain and loss can be coherently coordinated in the LH. However, not much is known about this LH inhibitory network due to its heterogeneous nature. Surprisingly, we found that LH GAD65 neurons are only partially overlapping with LH VGAT neurons and that ≈80% of LH VGAT neurons do not express GAD65. Interestingly, LH GAD65 neurons seem also distinct from NPY and LepRb neurons and thereby adding another component to the LH inhibitory network. Through chemogenetic modulation we found that LH GAD65 neurons display a different effect on body weight and energy expenditure compared to LH VGAT neurons which have been shown to promote weight gain. This apparent functional dichotomy was also reflected in their microcircuitry as LH GAD65 neurons seem to receive inhibitory input from LH VGAT neurons but not from other GAD65 neurons. Our data adds to a coherent understanding of hypothalamic circuitry essential for energy expenditure and compensation and thus opens possible avenues for further neurophysiological investigations of hypothalamic control of body weight.

Disclosures: C. Kosse: None. **D. Burdakov:** None.

Poster

731. Monoamines, Amino Acids, and Other Regulators of Energy Balance

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Topic: F.10. Food Intake and Energy Balance

Support: NIH Grant DA030425

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Title: Palatable diet cycling alters sensitivity to D-amphetamine

Authors: *C. F. MOORE, V. SABINO, P. COTTONE;
Lab. of Addictive Disorders, Departments of Pharmacol. and Psychiatry, Boston Univ., Boston, MA

Abstract: Eating disorders and forms of obesity are associated with brain reward dysfunction. In this study we investigated the sensitivity of the brain reward system of subjects undergoing chronic diet cycling by testing the effects of amphetamine, a dopamine releaser. For this purpose, a group of male Wistar rats was provided a regular chow diet 7 days a week (Chow/Chow), whereas a second group of rats was provided chow for 5 days a week, followed by a 2-day access to a highly palatable sucrose diet (Chow/Palatable). Following 5 weeks of diet alternation, we investigated amphetamine sensitivity during access to the palatable diet ('P Phase') as well as during withdrawal from it ('C Phase'). We measured the effect of amphetamine on locomotor activity and brain stimulation reward (BSR), home-cage self-administration of amphetamine, and amphetamine-induced conditioned place preference. Palatable diet cycling resulted in hypophagia of the standard chow, overeating of palatable food upon renewed access, and compulsive-like eating. During the P, but not the C phase, diet cycled rats showed decreased sensitivity to both the locomotor stimulating and the threshold-reducing effects of amphetamine. In addition, during access to Palatable diet, diet cycled rats showed increased self-administration of amphetamine in the home cage, as compared to controls. Finally, amphetamine-induced place preference was reduced in Chow/Palatable rats during the P Phase, as compared to control rats. These results indicate that diet cycled rats show a phase-dependent deficit in the brain reward system, as revealed by a decreased sensitivity to amphetamine when the highly palatable food access is renewed following withdrawal from diet cycling. In summary these results suggest that, in pathological eaters, brain reward dysfunction may be dependent upon the feeding state of the individuals.

Disclosures: C.F. Moore: None. **V. Sabino:** None. **P. Cottone:** None.

Poster

731. Monoamines, Amino Acids, and Other Regulators of Energy Balance

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Topic: F.10. Food Intake and Energy Balance

Support: NIH GM109811

NIH DK019302

Title: Fluoxetine administration during adolescence decreases chow intake via meal size but does not alter meal patterns later in life

Authors: *Y. TREESUKOSOL[1,2], S. D. IÑIGUEZ[3], T. H. MORAN[2,4];
[1]California State Univ. Long Beach, Long Beach, CA; [2]Dept. of Psychiatry and Behavioral Sci., Johns Hopkins Univ. Sch. of Med., Baltimore, MD; [3]Dept. of Psychology, Univ. of Texas El Paso, El Paso, TX; [4]Johns Hopkins Global Obesity Prevention Ctr., Johns Hopkins Univ., Baltimore, MD

Abstract: We have previously reported that exposure to fluoxetine (FLX), specifically during adolescence, results in altered sensitivity to mood-related stimuli in adulthood, as inferred by forced swim and social defeat paradigms. To further investigate how juvenile FLX exposure influences feeding later in life, we compared meal pattern parameters in male rats presented with a chow diet. Postnatal day (PD) 35 rats were injected with FLX (0 or 20 mg/kg) for 15 consecutive days (PD35-49). During adulthood, the animals were exposed to chronic variable stress (CVS) for 14 days (PD74-88). During drug treatment, FLX-exposed rats decreased intake by reducing meal size but displayed no significant changes in meal number. Consequently body weights were significantly lower than that of controls by PD40.No significant group differences in meal pattern parameters were observed after drug treatment completion (PD50), or in body weight by early adulthood (PD65). Furthermore, both groups responded similarly to a palatable diet (45% fat, 17% sucrose) presented during adulthood (PD74-88). Finally, no significant group differences in meal pattern parameters were observed following a CVS schedule. Whereas juvenile FLX administration results in enduring changes in mood-related measures, here, no long-term changes in ad libitum feeding are observed.

Disclosures: Y. Treesukosol: None. **S.D. Iñiguez:** None. **T.H. Moran:** None.

Poster

731. Monoamines, Amino Acids, and Other Regulators of Energy Balance

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Topic: F.10. Food Intake and Energy Balance

Support: USP-BS Grant CONS01/2015

Title: Clusterin, a proposed biomarker of food addiction, is upregulated in the Nucleus Accumbens of undernourished rats

Authors: C. RODRÍGUEZ-RIVERA[1], C. GONZÁLEZ-MARTÍN[1], E. LIZÁRRAGA-MOLLINEDO[2], E. FERNÁNDEZ-MILLÁN[2], C. ÁLVAREZ[3,2], F. ESCRIVÁ[3,2], *L. F. ALGUACIL[1];
[1]Univ. CEU San Pablo, Boadilla, Madrid, Spain; [2]Ciber de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM, ISCIII), Madrid, Spain; [3]Univ. Complutense, Madrid, Spain

Abstract: Clusterin is a multifunctional protein that seems to play a significant role in the control of eating behavior. Central administration of clusterin is followed by anorexia in rats, while ODN-induced downregulation results in increased food intake (Gil et al., Nature Commun 4: 1862, 2013). Interestingly, we have recently observed that serum clusterin correlates with loss of eating control in morbid obese patients (Rodríguez-Rivera et al., Eur Neuropsychopharmacol 25 Suppl 2: S628, 2015), a finding that led us to propose this protein as a potential biomarker of food addiction. Despite these antecedents, little is known about the biological role of clusterin within the brain reward system. As a first approach to increase this knowledge, we have quantified by immunoblotting the mitochondrial and cytosolic levels of clusterin in the Nucleus Accumbens (NAc) and Prefrontal Cortex (PFC) of female, Wistar undernourished rats, bearing in mind that malnutrition is known to increase both food and drug reward. Rats undernourished during fetal life and lactation (by restricting mother's access to food), and for 5 additional months after birth, overexpressed mitochondrial clusterin (2-fold) in the NAc, but not in PFC. Clusterin upregulation was not observed when undernourishment was limited to gestation and lactation and animals had free access to standard diet from weaning. Parallel and similar changes were seen in the NAc levels of fumarate hydratase, an enzyme previously related to brain reward alterations induced by either drugs of abuse, high fat foods or undernourishment. Further studies are highly recommended to understand the exact meaning of clusterin upregulation in terms of behavior.

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Poster

731. Monoamines, Amino Acids, and Other Regulators of Energy Balance

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Topic: F.10. Food Intake and Energy Balance

Support: WesternU Research Funds

Title: Emesis induced by L-type Ca^{2+} channel agonist FPL64176 in the least shrew (Cryptotis parva) is sensitive to Ca^{2+} channel inhibitors and is mediated by 5-HT_3Rs and NK_1Rs

Authors: W. ZHONG[1], S. CHEBOLU[1], *N. A. DARMANI[2];
[1]Basic Med. Sci., [2]Coll Osteo. Med. Pacific, Western Univ. Hlth. Sci., Pomona, CA

Abstract: Cytoplasmic calcium (Ca^{2+}) mobilization is an important factor in the induction of emesis. We have previously provided the first evidence that the selective L-type Ca^{2+} channel agonist FPL64176 is a fully efficacious emetogen in the least shrew at 10 mg/kg (i.p.). In this study we explored the potential mechanisms associated with this finding. As shown by c-Fos staining, FPL64176 (10 mg/kg, i.p.) activated the brainstem dorsal vagal complex (DVC) emetic nuclei including the area postrema (AP), nucleus tractus solitarius (NTS) and dorsal motor nucleus of the vagus (DMNX). Pretreatment with the L-type Ca^{2+} channel inhibitor nifedipine at 10 mg/kg dose (s.c.) 30 min prior to FPL64176 administration completely abolished FPL64176-induced vomiting and c-Fos introduction. In addition, FPL64176 led to enhancements of serotonin (5-HT) and substance P (SP) immunoreactivity in brainstem sections. We then confirmed the antiemetic potential of the selective serotonin type 3 receptor (5-HT_3R) antagonist palonosetron and the neurokinin 1 receptor (NK_1R) antagonist netupitant, indicating that FPL64176-elicited vomiting is mediated by both 5-HT_3R and NK_1R. Ca^{2+} release from intracellular stores through ryanodine receptors (RyRs) following voltage-dependent Ca^{2+} entry via LTCCs during neuronal depolarization plays an important role in amplifying cytosolic Ca^{2+} levels. Here we found that the RyR antagonist dantrolene (i.p.) attenuated FPL64176-evoked vomiting in a dose-dependent manner. Furthermore, a combination of ineffective doses of nifedipine and dantrolene exerted additive antiemetic effects. These findings demonstrate that FPL64176-induced emesis is dependent on Ca^{2+} mobilization involving extracellular Ca^{2+} through LTCCs and Ca^{2+}-permeable 5-HT_3Rs, as well as RyR-mediated Ca^{2+} release from intracellular stores, which consequently results in the elevation of the cytoplasmic free Ca^{2+} level, the fundamental mechanism for neurotransmitter release.

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Poster

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Title: Muscarinic, nicotinic and GABAergic receptor signaling differentially mediate fat-conditioned flavor preferences in rats.

Authors: *F. M. ROTELLA, K. OLSSON, N. MARTINEZ, A. MORDO, I. KOHEN, A. AMINOV, J. PAGIRSKY, A. YU, V. VIG, R. J. BODNAR;
Psychology, Queens Col. Dept. of Psychology, Flushing, NY

Abstract: Rats display conditioned flavor preferences (CFP) for fats with previous pharmacological analyses revealing that corn oil (CO)-CFP expression was mildly reduced by dopamine (DA) D1 or D2 as well as NMDA or opioid receptor antagonists. Acquisition of CO-CFP was eliminated by NMDA antagonists, and significantly reduced by DA D1 and DA D2, but not opioid antagonists. Muscarinic (scopolamine: SCOP) and nicotinic (mecamylamine: MEC) cholinergic receptor antagonists and $GABA_B$ (baclofen: BAC) receptor agonism reduced fructose-CFP expression, and SCOP, but not MEC or BAC eliminated fructose-CFP acquisition. The present study examined whether systemic administration of SCOP, MEC or BAC altered the expression or acquisition of CO-CFP. For CO-CFP expression, rats were trained over 10 sessions with a CS+ flavor in 3.5% CO and a CS- flavor in 0.9% CO. Two-bottle choice tests with CS+ and CS- flavors mixed in 0.9% CO occurred following vehicle, SCOP (1, 5, 10 mg/kg), MEC (1, 6, 8 mg/kg) or BAC (1.5, 3, 5 mg/kg). For CO-CFP acquisition, eight groups of rats received vehicle, SCOP (1 or 2.5 mg/kg), MEC (4 or 6 mg/kg), BAC (3 or 5 mg/kg) or a limited intake vehicle control 0.5 h prior to 10 CS+ and CS- training sessions followed by six 2-bottle CS+ and CS- choice tests in 0.9% CO. CO-CFP expression was significantly but marginally reduced by SCOP (5 mg/kg: 70%), MEC (8 mg/kg: 85%) and BAC (3 mg/kg: 74%) relative to vehicle (97-98%). CO-CFP acquisition was eliminated (41-59%) by SCOP at a dose of 2.5, but not 1, mg/kg, and was accompanied by a failure to observe CS+ and CS- intake differences during testing relative to vehicle (84-88%) and limited control (91-98%) conditions. In contrast, neither MEC nor BAC altered CO-CFP acquisition. These data implicate the muscarinic cholinergic receptor system in mediating acquisition (learning) of fat-induced preferences in the same manner that it affected acquisition of sugar-induced preferences.

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Poster

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Title: Evidence for Trace Amine-Associated Receptor 1 (TAAR1) as a novel target for compulsive binge-eating

Authors: A. D. HOWELL[1], A. FERRAGUD[1], M. C. HOENER[2], V. SABINO[1], *P. COTTONE[1];
[1]Departments of Pharmacol. and Psychiatry, Boston Univ. Sch. of Med., Boston, MA;
[2]Neuroscience, Ophthalmology and Rare Dis. Discovery & Translational Area, . Hoffmann-La Roche Ltd, Basel, Switzerland

Abstract: Binge eating of highly palatable food constitutes a core feature of some forms of obesity and eating disorders, as well as of the recently proposed disorder of food addiction. Binge eating episodes typically occur in short periods of time, resulting in a high eating rate, and are accompanied by loss of control over eating behavior. Trace amine-associated receptor 1 (TAAR1) is a poorly investigated G protein-coupled receptor expressed throughout the limbic system. Growing evidence suggests that TAAR1 agonism may represent a potential pharmacological treatment for addictive disorders. Here, we investigated the role of TAAR1 in food reward by testing the effects of the TAAR1 agonist RO5256390 on maladaptive feeding behaviors. For this purpose, male Wistar rats were trained to obtain a highly palatable, sugary diet (*Palatable* group) or a standard chow diet (*Chow* group) for 1 h a day, under a fixed ratio 1 (FR1) schedule of reinforcement. Following escalation and stabilization of food responding, the effects of RO5256390 were investigated on: *i*) binge-like eating of a palatable diet in the FR1 procedure, *ii*) excessive eating of a standard chow diet induced by food restriction; *iii*) eating rate measured as inter-food intervals (IFIs), *iv*) compulsive eating in a light/dark conflict test, *v*) anxiety-like behavior in a defensive withdrawal test, *vi*) food reward using a conditioned place preference test, and *vii*) food seeking behavior using a second-order schedule of reinforcement. Our results show that the TAAR1 agonist RO5256390 blocked binge-like eating in the *Palatable* group without affecting intake of the control *Chow* fed group. Interestingly, RO5256390 treatment did not decrease the excessive intake of a standard chow diet in food-restricted rats. In addition, the drug treatment selectively reduced the eating rate in binge-eating rats and fully

blocked compulsive eating, without affecting anxiety-like behavior in *Palatable* rats. Furthermore, administration of the TAAR1 agonist significantly blocked the rewarding effects of the palatable food in a conditioned place preference test, without affecting performance in controls. Finally, RO5256390 blocked food seeking of palatable food. Altogether, these results provide evidence for the involvement of TAAR1 in food reward and propose a novel pharmacological treatment for compulsive binge eating.

Disclosures: **A.D. Howell:** None. **A. Ferragud:** None. **M.C. Hoener:** A. Employment/Salary (full or part-time): Is an employee of F. Hoffmann-La Roche. **V. Sabino:** None. **P. Cottone:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Received RO5256390 from F. Hoffmann-La Roche.

Poster

731. Monoamines, Amino Acids, and Other Regulators of Energy Balance

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Program#/Poster#: 731.19/CCC13

Topic: F.10. Food Intake and Energy Balance

Title: Chronic high fructose corn syrup induces metabolic disorder and impaired dopamine function in the absence of obesity

Authors: *A. MEYERS, D. MOURRA, J. BEELER;
Queens Col., Flushing, NY

Abstract: The contribution of high fructose corn syrup (HFCS) to metabolic disorder and obesity, independent of high fat, energy rich diets, is controversial. While high fat diets are widely accepted as a model of diet induced obesity (DIO) and associated metabolic disorder, the value of HFCS alone as a rodent model of DIO is less clear. Though impaired dopamine function is associated with obesity, studies examining changes in dopamine function induced by HFD have yielded inconsistent results. The effect of chronic HFCS on the dopamine system has not been investigated. The objective of this study was to test the effect of both HFCS alone as well as HFCS combined with HFD to assess weight gain, glucose regulation, and evoked dopamine release using of fast-scan cyclic voltammetry (FSCV) in anesthetized mice. Male and female wild-type mice (C57/BL6) received either water or 10% HFCS solution (Best Flavors, HFCS-55) along with ad libitum chow for 15 weeks. Mice consumed an average of 13.5 mL/day of HFCS solution compared to water consumptions of 3.5 mL/day in control mice, contributing 4 kcal/day into their diets. Despite increased caloric load, no significant differences in body weight were observed. Mice consuming HFCS exhibit reduced glucose clearance (1 g/kg glucose tolerance test), less sensitivity to insulin (0.5 U/kg, insulin tolerance test), and reduced evoked dopamine

release in the dorsolateral striatum as measured by FSCV. We then tested the effects of adding HFCS to traditional HFD (Teklad TD. 06414, 60% calories from fat). Mice on combined HFD-HFCS exhibited pronounced weight gain with a greater than 60% increase in bodyweight over 15 weeks compared to controls whose bodyweight increased by only 21%. The HFD-HFCS mice exhibited glucose dysregulation and substantially reduced evoked dopamine release compared to controls. These data suggest that HFCS can induce metabolic disorder as well as altered dopamine function independent of weight gain. The inclusion of HFCS in standard HFD DIO model may better emulate current dietary conditions in Western cultures.

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Poster

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Topic: F.10. Food Intake and Energy Balance

Support: NIH Grant 5R01DC013904

Title: High dietary sugar and fat consumption are associated with microbiota dysbiosis and altered vagal gut brain communication

Authors: ***T. SEN**[1], B. T. IHDE[2], C. R. CAWTHON[3], A. HAJNALD[4], P. M. DILORENZOB[5], C. B. D. L. SERRE[3], K. CZAJA[2];
[1]Col. of Vet. Med. @ UGA, Athens, GA; [2]Dept. of Vet. Biosci. & Diagnos. Imaging, [3]Dept. of Foods and Nutr., Univ. of Georgia, Athens, GA; [4]Neural and Behavioral Sci., The Pennsylvania State University, Col. of Med., Hershey, PA; [5]Psychology, Binghamton Univ., Binghamton, NY

Abstract: While high fat (HF) diet consumption is well-known to play a central role in development of obesity, the low-fat/high-sugar diet was one of the major dietary advice in 1990s-as reflected by the USDA. On the contrary, recently a growing body of evidence has been pointing to the inadequacy of the "diet" solution for either weight loss or prevention of metabolic syndrome. However, little research has been done to understand the detrimental effect of LF diet on health. Our previous studies show that dysbiotic microbiota can alter gut-brain vagal communication and feeding behavior. Our present study investigate the effect of both HF and LF/high Sugar diet on microbiota composition, gut-brain communication and fat tissue accumulation leading to obesity. Specifically, we tested the hypothesis that both the HF and LF diet can alter the gut microbiota, induce gut inflammation and alter vagal gut-brain communication when compared to control chow diet. Sprague-Dawley rats were fed with either

HF, LF or control chow diet (RD) for 4 wks. Food intake, body weight and body composition were monitored daily and fecal samples were collected weekly. After 4 weeks, animals were sacrificed and gut, brain, liver and nodose ganglion were collected. We found that compare to RD both HF and LF fed rats significantly increased the amount of body fat. 16S rRNA sequencing showed a decrease in total bacterial density and an increase in Firmicutes abundance in both HFD and LFD fed rats, compare to RD. HFD and LFD fed rats exhibited an increase in cecum and serum LPS and expression of pro-inflammatory cytokines like IL-6, IL-1β and TNFα. Gastrointestinal vagal innervation and expression of tight junction protein occludin was decreased in both the HFD and LFD rats compare to RD. The analysis of the gut-brain communication showed the withdrawal of vagal afferents in the gut and nucleus of the solitary tract (NTS). Damaged peripheral nerve branches triggered activation of microglia in the nodose ganglia but not at the CNS areas containing motor perikarya and central afferent terminals of damaged fibers. Taken together, our study indicates that not only HFD but also consumption of low-fat/high-sugar diet induces dysbiosis of gut microbiota, increased gut inflammation, reduces gastrointestinal vagal innervation, disrupts tight junctions and alters vagal gut-brain communication that is associated with the obese phenotype.

Disclosures: **T. Sen:** None. **B.T. Ihde:** None. **C.R. Cawthon:** None. **A. Hajnald:** None. **P.M. DiLorenzob:** None. **C.B.D.L. Serre:** None. **K. Czaja:** None.

Poster

731. Monoamines, Amino Acids, and Other Regulators of Energy Balance

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 731.20/CCC14

Topic: F.10. Food Intake and Energy Balance

Support: NIH grant DK052849

Title: Diet-related differences in meal-induced synapsin-1 phosphorylation in the dorsal vagal complex.

Authors: *J. S. NASSE, R. C. RITTER;
Washington State Univ., Pullman, WA

Abstract: Meal size is directly controlled by gastrointestinal signals carried to the brain by vagal afferent fibers that synapse in the dorsal vagal complex (DVC) of the hindbrain. Peripheral vagal afferent endings are activated by gastric stretch and by gut peptides released in response to luminal nutrients. However, we recently reported that peripheral CCK or 4^{th} ventricle melanocortin-4 agonists both increase serine phosphorylation of the synaptic vesicle protein,

synapsin-1, in DVC vagal afferent endings. Phosphorylated synapsin-1 (pSyn-1) increases neurotransmitter release probability thereby increasing synaptic fidelity and strength. We also reported that DVC pSyn-1 levels are elevated after overnight feeding, and recently have observed a positive correlation between the caloric size of the last night-time meal and DVC pSyn-1 levels. Collectively, our observations indicate that DVC pSyn-1 levels may be modulated by both meal-related GI signals and central signals related to body energy status. To evaluate the role of meal-related signals on DVC pSyn-1, we subjected male rats to an overnight fast, and then re-fed them a 5g meal of chow (3.01 kcal/g), high-sucrose (3.85 kcal/g), or high-fat/high-sucrose (4.73 kcal/g) diet, and measured DVC pSyn-1 levels at several time points over a 4-hour period. We found that pSyn-1 was significantly elevated at 30 and 60 minutes after both chow and high-sucrose meals, but returned to pre-meal levels by 2 hours. Surprisingly, the high-fat meal failed into increase pSyn-1 at any time point. To assess the possibility that elevated pSyn-1 following the chow meal was mediated by activation of vagal afferent MC-4 receptors, we made 4^{th} ventricle injections of the MC-3/4 antagonist SHU9119 prior to a 5g chow meal. SHU9119 did not prevent the increase in pSyn-1 following the chow meal indicating that meal-related signals trigger increased pSyn-1 by mechanisms not dependent on MC-4 receptor signaling. Moreover, our results suggest that high-fat meals may be gram for gram less effective at increasing DVC pSyn-1 than low-fat meals. The mechanisms that mediate meal-related modulation of DVC pSyn-1 are under ongoing investigation. However, we speculate that reduced pSyn-1 following high-fat meals might contribute to over consumption and weight gain on high-fat diets.

Disclosures: J.S. Nasse: None. **R.C. Ritter:** None.

Poster

731. Monoamines, Amino Acids, and Other Regulators of Energy Balance

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Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

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Topic: F.10. Food Intake and Energy Balance

Support: Maurice Wilkins Centre

Title: Feeding and GLP-1 receptor activation stabilize β-catenin in specific hypothalamic nuclei in male rats

Authors: *D. R. GRATTAN[1], H. MCEWEN[1], S. LADYMAN[1], P. SHEPHERD[2];
[1]Univ. of Otago, Dunedin, New Zealand; [2]Univ. of Auckland, Auckland, New Zealand

Abstract: Polymorphisms in the TCF7L2 gene are associated with increased risk of type-2 diabetes and obesity. TCF7L2 is a co-factor that binds with β-catenin to promote gene transcription in the canonical Wnt/β-catenin pathway. Here, we investigated whether β-catenin/TCF signalling is regulated in the hypothalamus during the normal physiological response to food intake. Feeding acutely stablized β-catenin in neurons (increasing detectable cytoplasmic levels) in specific regions of the hypothalamus involved in metabolic regulation. Elevated β-catenin was associated with increased transcription of several TCF-responsive genes. The effect of feeding was mimiced by administration of the GLP1 agonist exendin-4, and was characterized by cAMP-dependent phophorylation of β-catenin at serine residues 552 and 675. The data suggest that β-catenin/TCF signalling might be involved in nutrient sensing in the hypothalamus, highlighting a potential role for altered hypothalamic function contributing to the risk of diabetes conferred by polymorphisms of TCF7L2.

Disclosures: D.R. Grattan: None. **H. McEwen:** None. **S. Ladyman:** None. **P. Shepherd:** None.

Poster

731. Monoamines, Amino Acids, and Other Regulators of Energy Balance

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Topic: F.10. Food Intake and Energy Balance

Support: Graduate School of Systemic Neurosciences

Title: Hedonic food overrides the efficacy of leptin effects on orexin neurons

Authors: *E. LEIDMAA[1,2], M. GAZEA[1], A. PISSIOTI[1], I. KALLO[3], B. LASZLO[3], Z. LIPOSITS[3], N. GASSEN[1], M. A. PHILIPS[4], A. PATCHEV[1], O. F. X. ALMEIDA[1];
[1]Max Planck Inst. of Psychiatry, Muenchen, Germany; [2]Grad. Sch. of Systemic Neurosciences-Ludwig-Maximilians-Universität, Muenchen, Germany; [3]Lab. of Endocrine Neurobiology, Inst. of Exptl. Medicine, Hungarian Acad. of Sci., Budapest, Hungary; [4]Dept. of Physiology, Inst. of Biomedicine and Translational Medicine, Univ. of Tartu, Tartu, Estonia

Abstract: Food is consumed to obtain energy but its sensory properties play an important role in the motivation to eat specific types of food. Eating that is driven by hedonic motivation poses a challenge to the homeostatic mechanisms that regulate energy balance. The aim of this work was to test how palatability might compete out the effects of leptin, a major satiety signal. We observed that exogenous leptin loses its efficacy to suppress feeding when mice are presented with a palatable food (PF) for a short period (1 h), indicating hedonic overriding of a potent

satiety signal. To explore the basis of this behavior, we focused on the lateral hypothalamus (LH) where leptin normally acts on orexin (OX) neurons to suppress appetite. We found that a short exposure to PF upregulates OX expression in both fasted and satiated mice. Interestingly, hedonic foods do not interrupt leptin signalling; on the other hand, OX neuron activity, as measured by c-fos expression, is not inhibited by leptin in mice exposed to PF. Subsequent experiments and analysis revealed the potential role of the galanin receptor 2 in mediating leptin actions on OX neurons. These findings suggest that interference with galanin-mediated activation of yet unidentified inhibitory inputs to OX neurons contributes to the overriding of leptin actions by PF. Alternatively, PF may increase the activity of other orexigenic circuits/factors that stimulate OX neurons to such an extent that they become refractory to the inhibitory actions of leptin.

Disclosures: **E. Leidmaa:** None. **M. Gazea:** None. **A. Pissioti:** None. **I. Kallo:** None. **B. Laszlo:** None. **Z. Liposits:** None. **N. Gassen:** None. **M.A. Philips:** None. **A. Patchev:** None. **O.F.X. Almeida:** None.

Poster

731. Monoamines, Amino Acids, and Other Regulators of Energy Balance

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Support: EC grant "Nudge-it" (607310)

Vetenskarådet

Title: Ghrelin changes food preference from high fat diet to chow in schedule-fed rats.

Authors: *S. L. DICKSON, E. SCHÉLE, T. BAKE;
Inst. of Neurosci. and Physiol., The Sahlgrenska Academy, Univ. of Gothenburg, Gothenburg, Sweden

Abstract: Ghrelin is a gut peptide released from the empty stomach that increases food intake. It has also been linked to food-related behaviours such as food motivation, food reward and food anticipatory activity. Ghrelin levels in the blood are linked to meal pattern, increasing prior to feeding. To mimic human meal eating behaviour in animals we used a scheduled feeding (SF) paradigm in which rodents have ad libitum access to chow and in addition 2h access to highly palatable high fat diet (HFD). Previous studies with this paradigm have shown that both rats and mice will rapidly adapt their feeding behaviour and as a result binge-eat on HFD. Here we

sought to investigate the role of ghrelin during binge-like meal eating induced by SF. We utilised a combination of two different animal models: pharmacologically manipulated rats via acute administration of ghrelin or genetically modified mice lacking the growth hormone secretagogue receptor 1A (GHS-R1A). For acute injections of either ghrelin or vehicle into the lateral ventricle (ICV) or intra-VTA, rats were surgically implanted with guide cannulas and then habituated to SF for at least 2 weeks prior to injections. GHS-R1A-KO mice and their wildtype (WT) littermates were scheduled-fed for 4 weeks. Remarkably and unexpectedly, we found that acutely injecting ghrelin ICV or intra-VTA resulted in a shift in food preference from high fat diet towards chow during the SF period without altering total daily energy consumption. However an increase of body weight was observed after ICV ghrelin. A fasting challenge also led to an increase in chow intake during the SF session but HFD intake did not reduce at the same time. GHS-R1A-KO mice were able to adapt and maintain large meals of HFD in a similar fashion as WT mice suggesting that the ghrelin signalling system may not have a critical role in acquisition or maintenance in this kind of feeding behaviour. In conclusion, ghrelin appears to act as a modulating factor for binge-like eating behaviour by shifting the food preference towards a healthier choice (from HFD to chow), effects that were clearly divergent from fasting.

Disclosures: S.L. Dickson: None. **E. Schéle:** None. **T. Bake:** None.

Poster

731. Monoamines, Amino Acids, and Other Regulators of Energy Balance

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Topic: F.10. Food Intake and Energy Balance

Support: NIH/NIDDK DK26687

SSMF

St. Luke's/Roosevelt Hospital Feasibility Grant

Title: Sleep restriction (4 h of sleep) impairs behaviorally-measured motivation to sham consume (sip and spit) a sweet-tasting beverage in healthy women

Authors: *H. R. KISSILEFF[1], M.-P. ST. ONGE[1], A. SHECHTER[1], P. S. HOGENKAMP[2], A. SCLAFANI[3];
[1]New York Obesity Nutr. Res. Ctr. - Med., Columbia Univ. Med. Ctr., New York, NY; [2]Dept. of Neurosci., Uppsala Univ., Uppsala, Sweden; [3]Psychology, Brooklyn Col. of CUNY, Brooklyn, NY

Abstract: Compared to habitual sleep (HS =9h), restricting sleep to 4 h per night (short sleep = SS) increases blood flow in response to food images in brain loci (AJCN 2012;95:818, 2012) that are active in animals when they encounter rewarding stimuli (Neuroscience 16.865, 2008). In order to test the hypothesis that SS may increase the reward value of, and motivation to consume, foods or beverages, that would lead to increased energy intake, a novel sham drinking sipping task, employing a sipometer (Kissileff, et al, SFN abstracts, 2010) was used. Seven, normal weight healthy women (mean BMI = 21.3 ± 0.5 kg/m^2) were tested on single trial days, after undergoing 3 nights of both SS and HS in randomized order. Sleep periods were separated by an interval of at least 4 weeks. Before each sleep period, they sampled 5 ml of sweet-tasting (10% sucrose equivalent aspartame) or non-sweetened cherry Kool-Aid®, and marked a picture of a cup that showed how much they would "want to drink" of each beverage. After each sleep condition, 7 h after eating a 500-kcal, high-fat, breakfast, they sham drank these beverages, pumped in response to pressure exerted on a straw (a behavioral measure of motivation), in four successive, counter-balanced, trials that consisted of 2 minutes of continuous reinforcement and unlimited time on a progressive ratio reinforcement schedule (PR) for each beverage. After HS, on the PR schedule, cumulative pressure difference between sweet and non-sweet beverage increased as the difference in amount wanted in the sampling test increased (slope = 0.33 PSI/mm ± 0.09, SE = 0.014, r^2 =0.73), but during SS, the slope was not significant. Therefore, SS blocked the behaviorally-measured motivational response of increased pressure difference between sweet and non-sweet beverage, during sipping on the PR schedule. On PR, after HS, sham intake difference increased as difference in post-beverage enjoyment rating (150 ml VAS, "none at all" to "extremely") increased, but after SS sham intake, the difference reversed and decreased (slope = 0.21 g/mm ± 0.16 SE) as difference in enjoyment rating increased. The difference in slopes between the two sleep conditions (0.53 g/mm ± 0.21 SE) was significant (p = 0.03). Therefore, opposite to the hypothesis, SS blunts or reverses the motivation to sham consume a sweet beverage. More data are needed to establish the conditional relationships among sleep patterns, brain activity, and motivated behavior in humans.

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Poster

731. Monoamines, Amino Acids, and Other Regulators of Energy Balance

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Title: Ire1α in Pomc neurons is required for thermogenesis and glycemia

Authors: *J. SUN[1,2], T. YAO[1,3], Y. GAO[1,4], Z. DENG[1,5], R. J. KAUFMAN[6], T. LIU[1], K. K. WILLIAMS[1];
[1]Div. of Hypothalamic Res., Univ. of Texas Southwestern Med. Ctr., Dallas, TX; [2]Dept. of Endocrinol., Zhujiang hospital, Southern Med. Univ., Gungzhou, China; [3]Dept. of Physiol. and Pathophysiology, Xi'an Jiaotong Univ. Sch. of Med., Xi'an, China; [4]Natl. Lab. of Med. Mol. Biol., Inst. of Basic Med. Sciences,Chinese Acad. of Med. Sci. and Peking Union Med. Col., Beijing, China; [5]Dept. of Obstetrics and Gynecology, the First Affiliated Hospital, Med. Sch. of Xi'an Jiaotong Univ., Xi'an, China; [6]Degenerative Dis. Program, Sanford Burnham Prebys Med. Discovery Inst., La Jolla, CA

Abstract: Whether neuronal inositol-requiring enzyme 1(*Ire1*) is required for the proper regulation of energy balance and glucose homeostasis is unclear. We found that pro-opiomelanocortin (*Pomc*)-specific deficiency of *Ire1α* accelerated diet-induced obesity concomitant with a decrease in energy expenditure. This hypometabolic phenotype included deficits in thermogenic responses to diet and cold exposure as well as 'beiging' of white adipose tissue. We also demonstrate that loss of *Ire1α* in *Pomc* neurons increased hepatic glucose production as well as impaired glucose and insulin tolerances. At the cellular level, deletion of *Ire1α* in *Pomc* neurons elevated hypothalamic ER stress and predisposed *Pomc* neurons to leptin and insulin resistance. Together, the current studies extend and confirm conclusions that *Ire1α*/*Xbp1s* and associated molecular targets link ER stress in arcuate *Pomc* neurons to aspects of normal energy and glucose homeostasis.

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Poster

731. Monoamines, Amino Acids, and Other Regulators of Energy Balance

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Title: Preoptic leptin function in energy homeostasis

Authors: *S. YU[1], H. CHENG[1], Y. HE[3], Y. XU[3], Y. JIANG[4], H. GAO[4], A. ZSOMBOK[4], A. V. DERBENEV[4], H. MÜNZBERG[2];
[1]Neurobio. of Energy Balance, [2]Central Leptin Signaling, Pennington Biomed. Res. Ctr. (PBRC), Baton Rouge, LA; [3]Pediatrics, Baylor Col. of Med., Houston, TX; [4]Physiol., Tulane Univ. Sch. of Med., New Orleans, LA

Abstract: Ambient temperature is a strong modulator of both energy expenditure and food intake. Activity of neural circuits controlling energy expenditure and food intake are concomitantly coordinated by thermosensory inputs to ensure the maintenance of energy balance under different temperature conditions. Neurons that express the long isoform of leptin receptor (LepRb) in the preoptic area (POA) are stimulated by warm ambient temperature and mediate warm-adaptive responses by suppressing energy expenditure and food intake. These same neurons are also involved in reproductive functions in female mice by regulating the surge of luteinizing hormone in response to leptin. The excitatory neurotransmitter glutamate is central in mediating warm responses by POA LepRb neurons, but it is not clear whether POA LepRb signaling plays a role during temperature-dependent adaptations. To investigate the function of leptin in POA LepRb neurons in energy homeostasis, LepRb was selectively deleted in the POA by stereotaxic injection of adeno-associated virus that expresses Cre in LepRb-flox mice ($LepRb^{POA}$ KO). 10-week-long follow-up studies after virus injection under either regular chow or high fat diet revealed no significant changes in body weight, food intake, and energy

expenditure in both male and female $LepRb^{POA}$ KO mice, compared to control virus-injected littermates (by repeated measures ANOVA). However, under high fat diet, the number of $pSTAT3^{+}$ cells in the POA was significantly correlated with the % body weight change at 10 weeks post injection (by linear regression, $r = -0.529$, $p < 0.05$). $LepRb^{POA}$ KO mice also maintained normal core temperature and showed no defect with adjusting the level of energy expenditure upon acute ambient temperature changes. Direct electrophysiological recording revealed heterogeneous effects of leptin (300nM) on POA LepRb neurons when presynaptic inputs were blocked (depolarization: 36%, hyperpolarization: 20%, no change: 44%), and this may explain the lack of leptin-induced c-Fos in POA LepRb neurons as a reflection of both inhibitory and stimulatory leptin effects. In summary, LepRb signaling in the POA uniquely contributes to body weight regulation only under a surplus energy state, in which the circulating leptin level is high. We are currently investigating the role of POA LepRb signaling during temperature-dependent adaptation of food intake.

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Poster

732. Neurocircuitry of Emotion: Amygdala and Ventral Hippocampus

Location: Halls B-H

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Topic: G.03. Emotion

Support: Pew grant

ONR MURI Grant N000141310672

Title: Exploring mechanisms for empathy-like behavior in rats

Authors: ***M. CONTRERAS**, K. G. CRUZ, J. CUMMINGS, A. HATFIELD, J.-M. FELLOUS; Psychology, Univ. of Arizona, Tucson, AZ

Abstract: Empathy is an affective response stemming from the understanding of another individual's emotional state, allowing one to react to social stimuli with appropriate pro-social behaviors. There is increasing support for the idea that a basic form of empathy (i.e. emotional contagion) is evolutionarily conserved and shared across mammalian species. Little is known, however, about higher forms of empathy or about its neurophysiological mechanisms. In humans, intranasal administration of oxytocin, a neuropeptide implicated in a number of social behaviors, increases empathy. Recent neuroimaging studies have also shown that observing

another's distress elicits activity in the anterior insular cortex. In this study we investigated whether the oxytocin system is necessary for the expression of empathic behavior using an operant rodent model of empathy. We next asked whether the neural activity of the rat anterior insular cortex correlates with the expression of empathy-like behavior. Finally, we assessed whether 22 KHz ultrasonic vocalizations, calls emitted by adult rats in aversive situations, were sufficient to produce empathy-like behaviors. Animals were trained to obtain food pellets by pressing either of two cued levers in an operant chamber. During the empathy test, one of the levers was programmed to also deliver a footshock (0.5 mA, 0.5 sec) to a conspecific animal which was placed in full view, in an adjacent chamber. Single lever (forced-choice trial) or both levers (free-choice trial) were cued throughout the course of testing. We observed that cerebro-ventricular administration of an antagonist of oxytocin disrupted the expression of empathy-like behavior, while the infusion of oxytocin exaggerated it. Electrophysiological recordings revealed that the neural activity of the anterior insular cortex decreased during the empathy test. We also observed that the playback of 22 KHz calls alone was generally sufficient to produce empathy-like behaviors in rats that were empathic toward a conspecific. These results give insights in the basic neurobiological basis of empathy and could be used to guide research on treatment of mental disorders in which empathy is deficient.

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Poster

732. Neurocircuitry of Emotion: Amygdala and Ventral Hippocampus

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Topic: G.03. Emotion

Support: Campbell Family Mental Health Foundation Operating Grant

Banting Postdoctoral Fellowship (Canadian Institutes for Health Research)

Title: Unpredictable chronic mild stress (UCMS) causes brain volumetric changes and network reorganization in a mouse model of depression

Authors: *Y. S. NIKOLOVA[1], K. A. MISQUITTA[2], J. ELLEGOOD[3], J. LERCH[3], M. BANASR[2], E. SIBILLE[2,4];
[2]Campbell Family Mental Hlth. Res. Inst., [1]Ctr. for Addiction and Mental Hlth., Toronto, ON, Canada; [3]Mouse Imaging Ctr., SickKids Hosp., Toronto, ON, Canada; [4]Dept. of Psychiatry, Dept. of Pharmacol. and Toxicology, Univ. of Toronto, Toronto, ON, Canada

Abstract: Human magnetic resonance imaging (MRI) studies have associated depression with alterations in the function, morphology and connectivity of a distributed corticolimbic circuitry including the amygdala, hippocampus, and prefrontal cortex. In parallel, stress-based rodent models have provided insight into the disorder's putative molecular mechanisms. To help bridge these complementary lines of research, we used MRI to assess regional brain volume and global structural covariance in the unpredictable chronic mild stress (UCMS) mouse model of depression. Male BALB/c mice were exposed to six weeks of UCMS and behaviorally characterized. High-resolution 7T structural MRI images were collected ex vivo from fixed brains (n=12 UCMS-exposed, n=9 controls). Volumetric comparisons between groups were carried out for 26 regions of interest (ROIs) pre-selected based on the human depression literature. Graph theory analysis using whole-brain (i.e., 155 ROIs) structural covariance metrics was applied to assess group differences in overall brain network organization. We evaluated mean network strength, mean shortest path length (indicative of global efficiency), and transitivity (local clustering), in addition to the degree (i.e., number of connections) and connection strength of the regions showing strongest volumetric effects in the previous analysis. Permutation testing across a range of density thresholds (5-40%) was applied to assess the significance of the observed between-group network attribute differences. UCMS-exposed mice showed volume increases in the amygdala, frontal association cortex, prelimibic cortex and medial orbital cortex, proportional to behavioral change ($p<0.05$). Furthermore, stressed animals showed a trend toward having a lower average network connection strength and transitivity in the 10-35% density range, concurrent with significantly higher degree and connection strength of the amygdala at densities greater than 20%. These changes occurred in the absence of any difference in shortest path length at any network density. No consistent differences in degree and connection strength emerged for the frontal association, prelimbic and medial orbital cortices. The increase in amygdala size and connectivity in UCMS is consistent with human work showing larger amygdala volumes in first-episode depression or following early life stress. The concurrent decrease in mean network connection strength suggests a global reorganization pattern favoring the selective strengthening of nodes involved in the avoidance of threat at the expense of other nodes of potential behavioral relevance.

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Poster

732. Neurocircuitry of Emotion: Amygdala and Ventral Hippocampus

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Program#/Poster#: 732.03/CCC23

Topic: G.03. Emotion

Title: Ventral hippocampus exerts a dynamic inhibitory influence on the behavioral and physiological responses to stress via the anterior hypothalamic nucleus

Authors: *J. BANG[1], J. KIM[1,2];
[1]Cells and Systems Biol., [2]Psychology, Univ. of Toronto, Toronto, ON, Canada

Abstract: Stress initiates the release of glucocorticoid hormones (GCs) by activating hypothalamic-pituitary-adrenal (HPA) axis which then triggers diverse adaptive physiological and behavioral responses. During emotionally stressful experience, the ventral hippocampus (vHPC) is believed to attenuate the HPA-axis activity by indirectly inhibiting the paraventricular nucleus of the hypothalamus (PVN). While much effort has been made to demonstrate the inhibitory influence of vHPC on the HPA-axis during psychogenic stress, the underlying neural pathway has not been directly examined. Using the pathway specific optogenetic approach in mice, we activated vHPC inputs at the anterior hypothalamic nuclei (AHN) during a 30 min-physical restraint stress and examined its effects on stress-induced anxiety behaviors in the elevated plus maze, the successive alleys, and open field tests. We also tested whether this input can change basal anxiety level by activating the same pathway in freely moving mice with no prior stressor during the same anxiety tests. Our findings suggest that the predominantly GABAergic AHN is the functional intermediary structure through which vHPC exerts its inhibitory control on both anxiety behaviors and physiological responses such as respiration and circulating corticosterone level. Together, these results show an important top-down modulation of stress response and anxiety level by the hippocampal-hypothalamic pathway as a key element in the central feedback of HPA-axis and inhibitory regulator of negative affect.

Disclosures: J. Bang: None. **J. Kim:** None.

Poster

732. Neurocircuitry of Emotion: Amygdala and Ventral Hippocampus

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Topic: G.03. Emotion

Support: Samuel N. Vodopia and Carol J. Hasson SURF Fellowship

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Title: Chronic stress alters fear behavior in a neuromodulator-dependent manner

Authors: *M. HUI, M. ZELIKOWSKY, B. YANG, D. J. ANDERSON;
Biol. and Biol. Engin., Caltech, Pasadena, CA

Abstract: Stress is a powerful experience that effects virtually all animal species. We tested the effects of delivering various forms of stress on subsequent fear behavior using an overhead looming stimulus assay as our behavioral readout. Interestingly, we found that various forms of chronic stress produce an increase in persistent fear responding to the looming stimulus, and that this effect is dependent on the type of chronic stressor employed. Given previous results in the laboratory indicating a role for various neuromodulators in the looming assay, we were interested in exploring the possibility of a larger role for neuromodulators in mediating chronic stress. Combining cre-mouse lines crossed to a fluorescent reporter mouse and applying CLARITY to map brain-wide neuromodulator expression, we found that chronic stress produces an increase in expression of various neuromodulators across brain regions known to encode stress and that this increase correlates with the degree of fear persistence produced by each chronic stressor. Consistent with these data, we found that various perturbations of these neuromodulatory systems in stressed mice was able to block this fear persistence, and that conversely, driving this system in unstressed mice was sufficient to induce persistent fear responding. Collectively, our data point to a broad role for neuromodulators in the regulation of chronic stress.

Disclosures: **M. Hui:** None. **M. Zelikowsky:** None. **B. Yang:** None. **D.J. Anderson:** None.

Poster

732. Neurocircuitry of Emotion: Amygdala and Ventral Hippocampus

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 732.05/CCC25

Topic: G.03. Emotion

Support: NIH R01 DA019921

Title: Chronic administration of glucocorticoid receptor ligands increases anxiety-like behavior and selectively increases serotonin transporters in the ventral hippocampus

Authors: *G. L. FORSTER, R. SOLANKI, J. SCHOLL, M. WATT;
Univ. South Dakota, Vermillion, SD

Abstract: Organic cation transporter-3 (OCT3) are involved in the clearance of monoamines in the brain, and are increased during amphetamine withdrawal in several limbic brain regions, including the ventral hippocampus, central nucleus of the amygdala and dorsomedial hypothalamus. Increased OCT3 expression in the ventral hippocampus in particular has been implicated in reduced extracellular serotonin in this region, leading to increased anxiety-like behavior in rats. However, the mechanism by which OCT3 expression is increased is not known. Expression of other organic cation transporters in peripheral tissue are increased by activation of

glucocorticoid receptors (GRs) and amphetamine increases the level of corticosterone. Therefore, we tested the hypothesis that corticosterone, acting via GRs, increases OCT3 expression in the limbic system. Male adult rats were treated for 2 weeks with daily injections of corticosterone (40 mg/kg) in the presence or absence of the GR antagonist mifepristone (20 mg/kg), with elevated plus maze (EPM) behavior, plasma and brains collected 24 hours following the last treatment. Corticosterone treatment decreased time spent in the open arms of the EPM and increased OCT3 receptor expression in the ventral hippocampus (but not amygdala, hypothalamus or dorsal hippocampus), although these effects were not blocked by GR antagonism. Interestingly, mifepristone in the absence of corticosterone treatment reduced plasma corticosterone levels, and increased serotonin transporter (SERT) and GR expression in the ventral hippocampus, with no effect in the amygdala, hypothalamus or dorsal hippocampus. Overall, findings suggest that corticosterone can selectively increase OCT3 receptor expression in the ventral hippocampus and increases anxiety-like behavior independent of actions upon GRs. This provides a mechanism by which amphetamine or chronic stress may alter monoamine reuptake to increase anxiety.

Disclosures: **G.L. Forster:** None. **R. Solanki:** None. **J. Scholl:** None. **M. Watt:** None.

Poster

732. Neurocircuitry of Emotion: Amygdala and Ventral Hippocampus

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Topic: G.03. Emotion

Support: NIH NIDA R25-DA033674

NIH NIDA R01-DA019921

Title: Differential effects of increased serotonin in the central vs. basal amygdala on anxiety-like behavior

Authors: *J. O. STERNBURG[1], A. AFZAL[2], J. SCHOLL[1], G. FORSTER[1];
[1]Basic Biomed. Sci., Univ. of South Dakota, Vermillion, SD; [2]Loyola Univ., Chicago, IL

Abstract: Anxiety arising during amphetamine withdrawal is associated with drug relapse. In a rat model of amphetamine withdrawal, increased anxiety-like behavior is observed alongside heightened stress-induced serotonin (5-HT) levels in the central nucleus of the amygdala (CeA) when compared to amphetamine-naive rats. Recent studies show that partial 5-HT depletion of basal amygdala (BA) that also encompassed the CeA reduces anxiety-like behavior in rats,

suggesting an anxiogenic effect of 5-HT in these regions. However, it is not known whether increased CeA 5-HT is directly related to anxiety-like behavior as observed during amphetamine withdrawal. To test this, bilateral cannula were implanted into the BA or CeA of adult male Sprague-Dawley rats. Following three days of acclimation to the infusion process, rats were bilaterally infused with either vehicle (0.5 µl per side) or paroxetine (0.5 µl of 0.5 µM per side) to discretely increase 5-HT levels. Forty minutes after the infusion, anxiety-like behavior was measured on the elevated plus maze (EPM). Paroxetine infusion into the CeA did not affect time spent in the open arms of the EPM as compared to vehicle-infused controls. However, infusion of paroxetine into the BA resulted in reduced time in the open arms of the EPM, indicative of increased anxiety-like behavior. Overall, the results show a causal relationship between BA but not CeA 5-HT and anxiety-like behavior and suggest differential roles of 5-HT in the expression of anxiety-like behaviors. Therefore, the effects of amphetamine withdrawal on serotonergic activity in the BA should be assessed as a potential locus for increased anxiety during withdrawal.

Disclosures: **J.O. Sternburg:** None. **A. Afzal:** None. **J. Scholl:** None. **G. Forster:** None.

Poster

732. Neurocircuitry of Emotion: Amygdala and Ventral Hippocampus

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Topic: G.03. Emotion

Support: NIH Grant MH099505

Title: Pharmacological inhibition of basolateral amygdala produces affective behavioral disruption of threat processing in rhesus macaques

Authors: *C. ELORETTE, P. A. FORCELLI, L. MALKOVA;
Georgetown Univ., Washington, DC

Abstract: The coordination of threat detection and appropriate behavioral responses involves diverse brain regions within the limbic system and midbrain. Evidence for a rapid-acting subcortical pathway for threat processing has been provided by studies of patients with cortical blindness who failed to showed a deficit in threat detection. This proposed pathway includes midbrain regions (superior colliculus), thalamic nuclei (pulvinar) and limbic structures (amygdala). Investigations into this pathway across species have suggested a role for the basolateral amygdala (BLA) in mediating the affective response to visual threats. Previous studies into this pathway have made use of aspiration or excitotoxic lesions, which produce

irreversible deficits and may damage nearby structures or tracts. We make use of a pharmacological approach, which allows precisely targeted reversible manipulation of a neural structure in an awake, behaving animal. We tested the hypothesis that input from the BLA mediates fearful defense behaviors in rhesus macaques when confronted with either naturalistic or socially relevant threats. Five juvenile male macaques were tested on a behavioral paradigm in which neutral stimuli, either a novel object or naturalistic video, and threatening stimuli, either taxidermy snakes, unfamiliar human, or unfamiliar conspecific, were presented. The animal's latency to retrieve a food reward from a tray above the stimulus was measured along with the time spent looking at the stimulus. Affiliative, defensive, or fearful behavioral responses displayed by the animal were also measured. Two of the animals were tested on this paradigm after receiving 9 nmol (1 μl at a concentration of 9mM) muscimol, a $GABA_A$ agonist, bilaterally to the BLA. Animals showed a significant ($p<0.05$) reduction in latency to retrieve the food reward from the threatening stimulus as compared to untreated animals [and as compared to their own, untreated baseline session] for both the naturalistic (snake) and socially relevant (unfamiliar macaque and human) stimuli. Inhibition of the BLA also produced an alteration in behavior directed towards social stimuli in this task as compared to untreated controls. These results suggest a role for the BLA as a critical mediator of affect and socially directed behavior in the proposed subcortical threat processing pathway.

Disclosures: C. Elorette: None. **P.A. Forcelli:** None. **L. Malkova:** None.

Poster

732. Neurocircuitry of Emotion: Amygdala and Ventral Hippocampus

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Topic: G.03. Emotion

Support: NIH/NIMH MH094360-01A1

NIH/NCI U01CA198932-01

Title: Novel subdivisions of the mouse anterior basolateral amygdalar nucleus (BLAa)

Authors: *H. HINTIRYAN, N. N. FOSTER, L. GOU, M. S. BIENKOWSKI, M. Y. SONG, M. ZHU, I. BOWMAN, S. YAMASHITA, A. W. TOGA, H.-W. DONG;
USC Stevens Neuroimaging and Informatics Inst., Univ. of Southern California (USC), Los Angeles, CA

Abstract: The anterior basolateral amygdalar nucleus (BLAa) forms part of the basolateral amygdala complex (BLA), which also includes the lateral amygdalar nucleus (LA) and the posterior (BLAp) and ventral (BLAv) basolateral nuclei. Most notably, the BLA is known for the role it plays in the acquisition and extinction of fearful memories. Its direct connections with medial prefrontal cortical areas involved in both fear acquisition and extinction bolster its role in the conditioned emotional response. We examined, in detail, the connections of the infralimbic (ILA) and prelimbic (PL) cortical areas with the BLA in C57Bl/6J male mice. As part of the Mouse Connectome Project (www.MouseConnectome.org), double co-injections of one anterograde (PHAL or BDA) and one retrograde (CTb or FG) tracer were made in the ILA (PHAL/CTb) and in the PL (BDA/FG). These coinjections clearly revealed the medial and lateral aspects of the BLAa. Coinjections in the different subdivisions of the BLAa confirmed these connections, revealed their laminar specific connectivity with the medial prefrontal cortex, and disclosed their connections with other structures throughout the brain. Tracer injections placed in different regions aside from the medial prefrontal cortex, including, but not limited to, the anterior cingulate cortex, agranular insular cortex, entorhinal cortex, caudoputamen, and olfactory tubercle, clearly demonstrated their unique connections with the medial or lateral BLAa, further validating the subdivisions. This structural segregation of the BLAa is indicative of its variegated functional relevance, potentially in different aspects of fear learning and extinction, which can be interrogated using different advanced technologies.

Disclosures: **H. Hintiryan:** None. **N.N. Foster:** None. **L. Gou:** None. **M.S. Bienkowski:** None. **M.Y. Song:** None. **M. Zhu:** None. **I. Bowman:** None. **S. Yamashita:** None. **A.W. Toga:** None. **H. Dong:** None.

Poster

732. Neurocircuitry of Emotion: Amygdala and Ventral Hippocampus

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Title: Entorhinal afferents drive feedforward inhibition in basolateral amygdala via distinct interneuronal populations.

Authors: *E. M. GUTHMAN[1], D. RESTREPO[2], M. M. HUNTSMAN[1];
[1]Dept. of Pharmaceut. Sci., [2]Dept. of Cell & Developmental Biol., Univ. of Colorado | Anschutz Med. Campus, Aurora, CO

Abstract: The basolateral amygdala (BLA) is a brain region that plays a vital role in associating specific environmental stimuli with emotionally salient valence information. Excitatory principal neurons (PNs) encode this integrated sensory-valence information (Herry et al, 2008; Paton et al, 2006; Shabel & Janak, 2009) and send this information to other brain regions to guide behavior (Ambroggi et al, 2008; Beyeler et al, 2016; Namburi et al, 2015; Stuber et al, 2011). However, inhibitory interneurons (INs) in the BLA gate the activity and plasticity of BLA PNs (Ehrlich et al, 2009; Bissière et al, 2003; Woodruff & Sah, 2007). One major source of feedforward inhibition to the BLA is the lateral entorhinal cortex (Brothers & Finch, 1985; Mouly & Di Scala, 2006), a region of both the olfactory and parahippocampal cortices associated with sensory object representation (Keene et al, 2016; Price, 1973; Tsao et al, 2013; Xu & Wilson, 2012). We hypothesized that entorhinal afferents to the BLA would target distinct populations of INs to drive feedforward inhibition of BLA PNs. To test our hypothesis, we performed whole-cell electrophysiology in horizontal slices of adult mice. To determine the role of entorhinal input in modulating BLA circuitry, we electrically stimulated lateral entorhinal cortex while recording from neurons in the BLA. Initial results demonstrate that entorhinal afferents reliably synapse onto parvalbumin positive INs (15 of 16 cells tested) to drive monosynaptic EPSCs (66.0 ± 11.6 pA, 7.2 ± 0.4 msec latency, 0.91 ± 0.23 msec jitter, n = 13 cells). These synaptic events are mediated by calcium permeable AMPA receptors (control EPSC: 57.6 ± 3.6 pA, 100μM Naspm: 44.1 ± 2.7 pA, p = 0.0085 Kolmogorov–Smirnov test, n = 7 cells, 67 control EPSC, 83 Naspm EPSC; rectification index = 0.089, n = 2 cells), suggesting that this synapse can undergo plastic changes (Mahanty & Sah, 1998; Nissen et al, 2010; Oren et al, 2009; Polepalli et al, 2010). Future experiments will test the hypotheses that entorhinal afferents target other populations of BLA INs and drive feedforward inhibition in BLA PNs.

Disclosures: E.M. Guthman: None. **D. Restrepo:** None. **M.M. Huntsman:** None.

Poster

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Topic: G.03. Emotion

Support: NHMRC

ARC

Title: Anatomy and physiology of the central extended amygdala.

Authors: *Y. SUN, S. HUNT, L. XU, P. SAH;
Queensland Brain Inst., The Univ. of Queensland, Brisbane, Australia

Abstract: Anxiety disorders represent the most common of psychiatric disorders, affecting nearly one in four adults in the population. The central nucleus of the amygdala (CeA), and its forebrain target the central sublenticular extended amygdala (SLEAc), two key parts of central extended amygdala, have been identified as critical elements of anxiety processing. We have studied the neuronal types and synaptic connections within this circuit using slice electrophysiological recordings in combination with tract tracing and *ex vivo* optogenetics in mice. Injection of retrograde tracers into the SLEAc revealed that the major projections from the CeA to the SLEAc originate in the lateral division of the CeA (CeL). We find that 78% of the retrogradely-labelled cells in the CeL expressed somatostatin (SOM). Using SOM-Cre mice we selectively expressed channelrhodopsin-2 (ChR2) in SOM+ neurons in the CeL. Whole-cell recordings from neurons in the SLEAc demonstrated that optogenetic activation of ChR2-expressing SOM+ terminals evoked inhibitory synaptic responses in most SLEAc neurons. To test reciprocal connections, ChR2 was expressed in neurons in the SLEAc and whole-cell recordings were obtained from CeL neurons in acute slices. Photo-activation of terminals in the CeL shows that the SLEAc sends feedback projections to the CeL, which is largely GABAergic. Together, these results define a reciprocal inhibitory circuit between CeL and SLEAc, which prepare the ground to dissect the functional circuitry of CeL-SLEAc and to examine its behavioral role in anxiety.

Disclosures: **Y. Sun:** None. **S. Hunt:** None. **L. Xu:** None. **P. Sah:** None.

Poster

732. Neurocircuitry of Emotion: Amygdala and Ventral Hippocampus

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Topic: G.03. Emotion

Support: CIHR

NSERC

CNPQ

Title: Role of the ventral hippocampal projections to the lateral septum in fear and anxiety

Authors: *G. M. PARFITT, J. BANG, R. NGUYEN, A. AQRABAWI, J. KIM;
Dept. of Psychology, Univ. of Toronto, Toronto, ON, Canada

Abstract: Anxiety behaviour is shaped by a distributed network of forebrain limbic structures, including the septum and the hippocampus. In particular, neural inputs arising from the ventral hippocampus (vHPC) to the lateral septum (LS) are thought to serve as a key component of the anxiety circuit. In the present study, we investigated the behavioural contribution of the vHPC-LS pathway in modulating anxiety-related behaviours in mice. We targeted the LS-projecting vHPC neurons by injecting the retrogradely propagating CAV2-Cre into the LS and injecting Cre-responsive AAV (AAV-hSyn-DIO-hM3D (for activation) or hM4D (for inhibition) into the vHPC. After CNO injection, the animals were submitted to behavioural test paradigms for anxiety: elevated plus maze (EPM), and open field (OF), novelty feeding suppression test (NFST) and fear conditioning (FC). The activation of LS-projecting vHPC neurons using hM3D increased the open arm time in the EPM without changing the locomotor activity. Similarly, in the NFST CNO-treated hM3D mice displayed reduced latency to eat in a novel anxiogenic environment with no effect on food intake in the home cage. In the OF, no alterations were observed in both locomotor activity and center time. In contrast, the inhibition of LS-projecting vHPC neurons using hM4D decreased the open arm time and centre time during the EPM and the OF, respectively. In the FC, the activation of the LS-projecting vHPC neurons during the fear acquisition reduced the freezing level 24h after the acquisition in the hM3D mice. However, the inhibition of the same neuron population did not alter the freezing level in the hM4D mice. In summary, our findings demonstrate that the LS-projecting vHPC neurons modulate anxiety-related behaviours in a bidirectional manner, and their activation can reduce contextual fear memory expression.

Disclosures: G.M. Parfitt: None. **J. Bang:** None. **R. Nguyen:** None. **A. Aqrabawi:** None. **J. Kim:** None.

Poster

732. Neurocircuitry of Emotion: Amygdala and Ventral Hippocampus

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Topic: G.03. Emotion

Support: NSERCC Grant A7077

CIHR Grant

Title: Maternal circuits that respond to mouse pup vocalizations: D2 dopamine and oxytocin receptors

Authors: *J. S. YEOMANS[1], B. J. PEREIRA[2];
[2]Psychology, [1]Univ. Toronto, Toronto, ON, Canada

Abstract: Social communication and bonding are associated with dopamine, oxytocin and prolactin signals in many mammalian species. Ultrasonic vocalizations (USVs) in isolated 3-11 day old rodent pups increase maternal behaviors and hormonal responses: retrieval, licking and grooming, nursing, prolactin and oxytocin. Oxytocin projections from hypothalamus to dopamine neurons and nucleus accumbens shell are important for social attachments in rats. For example, blockade of D2 or oxytocin receptors in nucleus accumbens shell reduces maternal responses to pups, and memories of pups (D'Cunha et al., 2011). Oxytocin and D2 receptors are colocalized as heteromers in nucleus accumbens shell where both are critical for maternal responses (Romero-Fernandez et al., 2013). In mice, D2 dopamine receptor gene deletion reduces pup USVs, and maternal retrieval responses and prolactin surges to pup USVs (Curry et al., 2013). Both pup and dam D2 genes influence the number of USVs by pups, as shown by heterozygous and reciprocal crosses, respectively. Oxytocin receptor gene deletions similarly reduce pup USVs and maternal responses to pups (Hidema et al., 2015). Maternal responses to pup USVs depend on the dam's left auditory cortex, where oxytocin receptors improve neuronal sensitivity to pup USVs (Marlin et al., 2015). Oxytocin and vasopressin receptors on olfactory, amygdala and entorhinal cortex neurons are relevant to social recognition and memory. Entorhinal cortex receives sensory information from auditory, olfactory and visual cortex (all with oxytocin receptors), and projects to hippocampal CA2 and CA3 pyramidal neurons with oxytocin receptors. Oxytocin receptors on medial periaqueductal gray neurons needed for vocalizations may improve pup USV production. Therefore, when dams retrieve pups after USVs, oxytocin production in the dam's hypothalamus may facilitate maternal responses via dopamine output, pup recognition, bonding and memory via oxytocin neurons that project to olfactory, auditory, mesolimbic, amygdala, entorhinal and CA2/3 hippocampal circuits.

Disclosures: J.S. Yeomans: None. **B.J. Pereira:** None.

Poster

732. Neurocircuitry of Emotion: Amygdala and Ventral Hippocampus

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Title: Use awake rat fMRI to visualise fear memory and weakened fear memory representations in the brain

Authors: A. KOLESNYK[1], A. P. HARRIS[2], M. C. HOLMES[3], *S.-H. WANG[4,5];
[1]Ctr. for Clin. Brain Sci., [2]Ctr. for Cognitive Ageing and Cognitive Epidemiology, [3]Ctr. for Clin. Brain Sciences, Cognitive Ageing, Cadiovascular Sci., [5]Ctr. for Clin. Brain Sciences, Ctr. for Cognitive Ageing and Cognitive Epidemiology, [4]Univ. of Edinburgh, Edinburgh, United Kingdom

Abstract: Functional magnetic resonance imaging (fMRI) is an invaluable and non-invasive tool for visualising the emotional and cognitive processes in the brain at the systems level. While mostly used in humans, recent studies have made advancement in using fMRI in awake rodents to investigate learned behaviours. For example, rodent fMRI has been used to map activated brain regions involved in normal, stressed, and genetically modified animal models. This provides significant translational value, such as development of pre-clinical models to enhance the understanding of brain functions at the network level and to provide biomarkers for drug discoveries. Previous studies have investigated the brain circuits involved in visual fear conditioning by comparing the circuits in conditioned and unconditioned animals. However, several important questions remain to be answered. First, whether fearful memories from different sensory domains activate similar brain circuits or are domain specific, and does the brain represent fearful and neutral memories differently? Second, which brain areas show activity changes when the fear memory is weakened? To answer the first question, we designed an olfactory fear conditioning paradigm, which allowed the rats to associate one odour with the fear while a second odour remained neutral. We found that the rats showed significantly greater freezing when presented with the conditioned odour, compared to the neutral one. They underwent brain imagining in the 7T MR scanner. The results showed the BOLD acyivation signals were stronger in the amygdala, perirhinal and somatosensory cortex when the animals were exposed to the fearful odour than when they were exposed to the neutral odour. To answer the second question, we treated rats with a beta-adrenergic blocker, propranolol, shortly after fear memory reactivation. This has been shown to be an effective method to weaken the persistence of fear memory by blocking memory reconsolidation. The propranolol-treated rats showed significant reduction of conditioned fear responses and significant reductions in brain activitation compared to control rats. Together, we identified similar fear circuits for fear-conditioned stimuli across different sensory domains. Activity changes in these key areas can be used as biomarkers for identifying effective drug targets for treating fear-related disorders.

Disclosures: **A. Kolesnyk:** None. **A.P. Harris:** None. **M.C. Holmes:** None. **S. Wang:** None.

Poster

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Topic: G.03. Emotion

Support: NIH Grant 5R21DA035144-02

Title: Dissecting the role of extended amygdala input to the locus coeruleus in motivated behaviors

Authors: *D. L. BHATTI[1,3], M. R. BRUCHAS[2];
[2]Div. of Biol. and Biomed. Sciences, Dept. of Neuroscience, Anesthesiol., [1]Washington Univ. in St. Louis, Saint Louis, MO; [3]Div. of Biol. and Biomed. Sciences, Dept. of Neuroscience, Anesthesiol., Washington Univ., Saint Louis, MO

Abstract: Both the Bed Nucleus of the Stria Terminalis (BNST) and the Locus Coeruleus (LC) are thought to play integral roles in processing stressful stimuli to subsequently elicit suitable behavioral responses. The heterogeneous neuronal populations of the BNST have been shown to influence motivated behaviors related to stress, and LC activity is critical for stress-induced behavioral responses. However, how these regions interact to modulate motivated behavior is unknown. To dissect the interaction between these neuronal populations, we first characterized a dense BNST GABA/CRFergic projection to the LC using anterograde and retrograde viral tracing. Using an *in vivo* optogenetic approach, male VGAT-cre mice were injected with a cre-dependent channelrhodopsin-2 virus (DIO-ChR2-eYFP) in the BNST to selectively target GABAergic neurons. We then implanted optic fibers above the LC to specifically activate GABAergic BNST terminals in the LC region. Activation of BNST-LC^{VGAT} terminals produced frequency-dependent real-time place preference and optical self-stimulation compared to controls. These data suggest that the BNST may send GABAergic inputs which inhibit LC neurons thereby suppressing norepinephrine release to efferent regions to ultimately elicit positively-motivated behavioral responses. We are currently using electrophysiological and pharmacological techniques concurrent with BNST-LC^{VGAT} photostimulation to characterize and examine the receptors modulating LC activity and *in vivo* Ca^{2+} imaging to visualize the network dynamics of LC-projecting BNST neurons.

Disclosures: D.L. Bhatti: None. **M.R. Bruchas:** None.

Poster

732. Neurocircuitry of Emotion: Amygdala and Ventral Hippocampus

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Program#/Poster#: 732.15/DP09 (Dynamic Poster)

Topic: G.03. Emotion

Title: Functional mapping of central amygdala feeding and reward circuits

Authors: *H. KUCUKDERELI[1,2,3], A. M. DOUGLASS[1,2], M. PONSERRE[1], J. GRÜNDEMANN[4], M. MARKOVIC[4], P. L. ALCALA MORALES[1], C. STROBEL[1], A. LÜTHI[4], R. KLEIN[1];
[1]Max Planck Inst. For Neurobio., Martinsried, Germany; [2]Grad. Sch. of Systemic Neurosciences (GSN), Ludwig-Maximilians-University(LMU), Martinsried, Germany; [3]Intl. Max Planck Res. Sch. for Mol. and Cell. Life Sci. (IMPRS-LS), Martinsried, Germany; [4]Friedrich Miescher Inst., Basel, Switzerland

Abstract: The central nucleus of the amygdala (CeA) is commonly associated with processing negative valance signals to execute aversive behaviour in the context of learnt fear and anorexia. Neurons within the mouse CeA that express protein kinase C-δ (PKCδ) induce feeding suppression under satiety and malaise conditions and when unpalatable foods are encountered. The function of these cells is proposed to be mediated by local inhibitory interactions within the CeA. We have identified a population of PKCδ-negative neurons in the CeA that express Htr2a, which promote food intake and reward related behaviours. We have used electrophysiological and virus-mediated methods to map and test the functionality of the circuits in which $CeA^{PKC\delta}$ and CeA^{Htr2a} function to antagonistically control food intake.
Use of mono-synaptic rabies tracing and ChR2-assisted circuit mapping revealed that $CeA^{PKC\delta}$ and CeA^{Htr2a} neurons reciprocally inhibit each other. We also found that CeA^{Htr2a} and $CeA^{PKC\delta}$ neurons sit within a network of brain regions that have described roles in food intake and energy homeostasis. These neurons also send axons to multiple brain regions including lateral hypothalamus, lateral parabrachial nucleus (lPBN), nucleus of the solitary tract and medulla. ChR2-assisted circuit mapping revealed strong inhibitory connections from CeA^{Htr2a} neurons to lPBN and brain stem. Together, these data suggest that the CeA influences feeding behaviour through both local inhibitory connections and long range outputs. Our study has demonstrated that not only is the CeA important for mediating aversive behaviours but this region also processes appetitive signals to promote reward-related behaviours.

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Poster

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Program#/Poster#: 732.16/DDD9

Topic: F.01. Neuroethology

Support: SFN Early Post Doc mobility

Title: The role of amygdala-striatal circuitry in exploration

Authors: *P. BOTTA[1], M. ANGELHUBER[3], M. VICENTE, 1400-038[2], R. COSTA[2]; [1]Neurobio., [2]Champalimaud Fndn., Lisbon, Portugal; [3]Bernstein Ctr. Freiburg, Freiburg, Germany

Abstract: In a world varying in time and space, exploration of new territories can increase inclusive fitness. Exploratory activity is achieved through the progression of a stochastic selection of motor actions essential to explore and become familiarized with novelty. Such actions are modulated by emotional states. The inability to cope with variable novel situations and environments is symptomatic of serious emotional disorders such as anxiety and depression in humans. Given that the initiation of specific exploratory actions is sensitive to contextual and emotional states, we decided to explore the contribution of limbic brain areas to exploratory activity. The amygdala is a well-known limbic structure important for emotional processing that projects to the medial parts of striatum involved in action selection. Therefore, we investigated the role of amygdala-striatal circuits in action selection computation during exploration. We developed a dimensionality emergence assay to investigate differences in exploratory activity in a habitual and novel environment, and during the process of habituation. In order to specifically target the basolateral portion of amygdala, we characterize a transgenic mouse line specifically expressing CRE recombinase in basolateral amygdala. We found that it expresses in pyramidal calmodulin kinase II expressing neurons. Using this line, we imaged the Cre defined amygdala neuronal ensembles in freely-exploring mice using conditional viral expression of calcium-sensors in the paradigm described above. Principal cells of basolateral amygdala massively project to dorsomedial striatum. The effect of amygdala-striatal projections on locomotor activity and the functional connectivity with dopamine receptor 1 and 2 expressing neurons is currently being tested using optogentic tools combined with slice electrophysiology. These experiments will increase our understanding of the role of a key limbic circuit on processing different contextual and emotional information to favor exploratory motor actions.

Disclosures: **P. Botta:** None. **M. Angelhuber:** None. **M. Vicente:** None. **R. Costa:** None.

Poster

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Topic: G.03. Emotion

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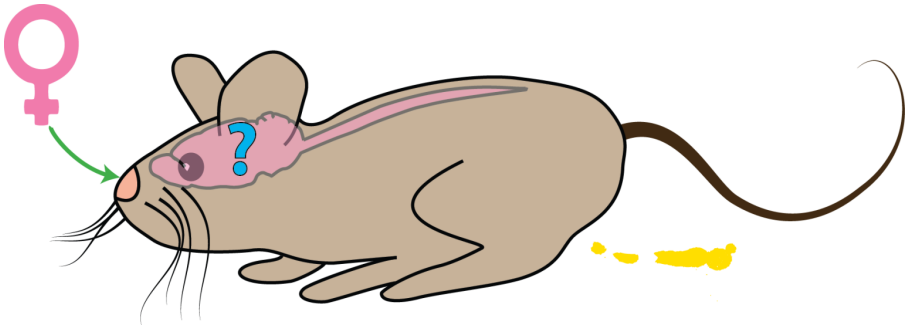
NSF-GRFP DGE-1144086

Title: To pee or not to pee: defining a limbic circuit from nose to bladder

Authors: ***J. A. KELLER**[1], L. STOWERS[2];
[1]Neurosciences, UC San Diego, La Jolla, CA; [2]The Scripps Res. Inst. / UC San Diego, La Jolla, CA

Abstract: Male mice without previous female experience will reliably countermark (e.g. urinate, micturate) when presented with female urine. We aim to unravel the necessary path that olfactory information must travel to reach the urinary motor system, which is relatively simple at the muscular level. To this end, we have identified the brainstem premotor neurons necessary for this behavior, as well as traced their inputs using modified rabies virus. Because these neurons are embedded in a larger network of autonomic function, precise manipulations using optogenetic and chemogenetic techniques have been necessary to probe animals performing this natural behavior. We hope that the combination of a molecularly-defined input and low-dimensional output in this circuit will ultimately allow a system-level approach to interrogate the function of every synapse from nose to bladder, in order to reveal mechanisms of innate behaviors in mammals.



Disclosures: **J.A. Keller:** None. **L. Stowers:** None.

Poster

733. Emotion: Information Processing

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 733.01/DDD11

Topic: G.03. Emotion

Support: CONACyT 155520

Title: Do women exhibit greater emotional context effect on eye movements inhibition control?

Authors: A. ABUNDIS[1], R. V. HUERTA[2], *J. RAMOS-LOYO[3], E. MATUTE[1];
[1]Inst. de Neurociencias, [2]Psicología, [3]Univ. de Guadalajara, Guadalajara, Mexico

Abstract: Several studies have shown that an efficient inhibitory control promotes emotional regulation. Accumulating evidence reveals that emotional stimuli have an impact on cognitive processes, either interfering or improving performance in cognitive tasks. Additionally, differences between men and women have been reported in different studies, pointing out differences in strategies used and underlying neural resources during cognitive and emotional processing. The goal of the present study was to identify sexual differences in the effect of emotional stimuli on inhibitory control recording eye movements during a Go/No-go task. Forty young adults (20 women) participated in the study. Participants had to press a key when the arrow in the center of the screen was congruent in color and direction with the bar presented on the left or right side of the screen (Go trials, 75%). However, when those conditions were not met participants must avoid responding (No/Go trials, 25%). Four conditions were included, two non-emotional (no picture, neutral) and two emotional backgrounds (pleasant and unpleasant). Eye movements were recorded using a Tobii eye tracker. Three regions of interest were defined to measure both number of fixations and duration of each fixation. Behavioral data also included reaction time and inhibition errors. Results indicated that the unpleasant context caused a higher number of inhibition errors. Besides, a higher percentage and longer duration of fixations in the arrow than in the surrounding area was observed under the non-emotional conditions than the emotional ones and, the opposite pattern was evident under the emotional conditions. Women showed a higher percentage and longer duration of fixations in the surrounding area than men at the neutral and emotional contexts. In sum, present results confirm the assumption that emotional pictures receive preference attention compared with neutral ones generating more difficulties in inhibition processing. This hindering effect is higher in women than in men.

Disclosures: **A. Abundis:** None. **R.V. Huerta:** None. **J. Ramos-Loyo:** None. **E. Matute:** None.

Poster

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Topic: G.03. Emotion

Support: NIMH IRP

Title: Monkey face-selective regions differ in their ability to classify facial expressions

Authors: *M. FLESSERT, H. ZHANG, L. G. UNGERLEIDER;
Lab. of Brain and Cognition, NIMH/NIH/DHHS, Bethesda, MD

Abstract: In our prior work (Zhang et al. 2016) we reported that face-selective regions in the human brain differ in their ability to classify facial expressions. Monkeys, like humans, can discriminate and classify different categories of expression automatically and effortlessly. Yet the ability of the various face-selective regions ('face patches') in the monkey brain to discriminate between different facial expressions remains unclear. To explore the underlying neural computations for this ability across the two primate species, we performed a parallel study on macaque monkeys. Here we used fMRI and support vector machine (SVM)-based multi-voxel pattern analysis to examine the ability of macaque face-selective regions to discriminate among different categories of monkey facial expressions.

Three male macaques were injected with MION before fMRI scanning. A slow event-related fMRI experiment was performed in which monkeys viewed 32 monkey face images belonging to 8 different identities and portraying 4 different expressions: fear grin (fearful), threat (aggressive), lip smack (appeasing) and neutral. In separate localizer runs monkeys viewed blocks of monkey faces, objects and scrambled images.

A GLM contrast of faces>objects was used to localize the monkey's face patches in and around the superior temporal sulcus (STS): middle fundus, middle lateral, anterior fundus and anterior lateral. The contrast of faces>scrambled images localized face processing in the amygdala. A GLM of each monkey face was created and the beta-values were extracted for each face patch and the amygdala. A one-versus-others SVM and leave-one-identity-out cross-validation were performed to obtain the decoding accuracy rate.

We found that the decoding accuracy was significant for discriminating fear grin relative to all other expressions in the amygdala. For the face patches in the fundus of STS, the decoding accuracy was significant for discriminating neutral expressions relative to all emotional expressions. These results are comparable to those obtained our earlier study in humans (Zhang et al. 2016), where we found the human amygdala to be significant at discriminating fearful faces from other expressions and the right STS to be significant at discriminating neutral faces from all emotional expressions.

Taken together, our findings suggest that the different face-selective regions in the monkey brain make distinct contributions to the processing of the emotional content within faces. In addition, the monkey fundus face patches may play a role analogous to the human STS in discriminating neutral faces from emotional ones.

Disclosures: M. Flessert: None. **H. Zhang:** None. **L.G. Ungerleider:** None.

Poster

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Topic: G.03. Emotion

Support: NMRC/CBRG/0088/2015 (Singapore)

Title: Task congruence of disgust expression modulates brain activation and connectivity in cognitive control regions during face judgment

Authors: *E. NG[1], Y.-J. WANG[1], J.-H. POH[1], J. DE SOUZA[1], J. LIM[1], Y.-T. HAN[1], C.-Y. CHEN[1], J. ZHOU[1,2];
[1]Neurosci. and Behavioral Disorders Program, Duke-NUS Med. Sch., Singapore, Singapore;
[2]Clin. Imaging Res. Center, A*STAR-National Univ. of Singapore, Singapore, Singapore

Abstract: Emotional facial expressions capture attention [1]. The perceptual or valence nature of the capture is still debated [2]. With a widely used face judgment task, we hypothesized that, while cognitive control regions should be recruited to maintain task performance [3], a capture by valence should activate additional disgust-related regions more consistently than a perceptual one.
Methods
Young adults (N = 44, 18 F) judged the expression (neutral or disgust) or gender of faces in two separate experimental blocks in one fMRI session (3T, TR 2s). Behavioral data were subject to two-way repeated measures ANOVA (2 expression: neutral or disgust x 2 task: emotion or gender). Similarly, we analyzed brain activation using general linear model and functional connectivity using psychophysiological interaction (PPI; SPM8).
Results
Participants were accurate in both tasks (> 88%), higher in emotion compared to gender condition ($p < .001$). Response to disgust faces was faster than that of neutral faces during emotion judgment but the pattern reversed during gender judgment (interaction $p < .001$). Disgust expression captured attention, with effect contingent on task congruency.

Interaction effect in activation strength (height $p < .001$, extent $p < .05$, unc.) was observed in bilateral insula, right inferior frontal gyrus (IFG), left precentral gyrus (PreCG), and right angular gyrus (ANG). Activation was greater in disgust than neutral in gender condition while the opposite was observed in emotion condition. Insula is implicated in salience detection and perceptual evidence accumulation [4], while precentral and angular gyri are involved in cognitive control [5]. PPI analysis yielded similar expression-by-task interaction on the connectivity between left PreCG and bilateral inferior parietal sulci, and that between right ANG and left PreCG and IFG orbital areas, suggesting connectivity changes only in cognitive control regions [5].

Conclusion

Attention recruitment was less when expression was task-congruent (emotion), but more when incongruent (gender), which may be due to compensation for the capture. The circuit of disgust was not particularly engaged, suggesting that the capture may be perceptual in nature. Participants might need only the visual facial features [2] for quick decisions even when valence information was available. Despite automatic in its processing, the use of emotional information is modulated by current goal and context [6].

References

[1] Hodsoll et al., 2011, *Emotion*
[2] Nummenmaa & Calvo, 2015, *Emotion*
[3] Jasinska et al., 2012, *Front Psychol*
[4] Pedersen et al., 2015, *PLoS ONE*
[5] Kohn et al., 2014, *NeuroImage*
[6] Pessoa, 2009, *Trends Cogn Sci*

Disclosures: E. Ng: None. **Y. Wang:** None. **J. Poh:** None. **J. de Souza:** None. **J. Lim:** None. **Y. Han:** None. **C. Chen:** None. **J. Zhou:** None.

Poster

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Kavli Foundation

NERF/VIB

Title: Studying the function and connectivity of habenular networks in zebrafish brain

Authors: *E. YAKSI[1], E. BARTOSZEK[2], S. KUMAR JETTI[3], S. FORE[2];
[2]Kavli Inst. for Systems Neurosci., [1]Norwegian Univ. of Sci. and Technol., Trondheim, Norway;
[3]MIT, Boston, MA

Abstract: The habenula (Hb) is a brain region with increasing popularity due to its strong link to addiction, mood disorders and experience dependent fear. We demonstrated that Hb neurons respond to odors and light asymmetrically. Moreover, we showed that Hb neurons exhibit structured spontaneous activity that is spatially and temporally organized. This spontaneous activity resembles neural attractors, which can switch the preferred state of the Hb and regulate the transmission of sensory information to downstream monoaminergic brainstem nuclei. In order to explore the source of Hb spontaneous activity, we investigate the local connectivity within Hb and the global functional inputs to Hb. We showed that recurrent excitatory connections within Hb is important for maintaining spatio-temporal organization of Hb activity. Moreover, we observed that functional inputs form zebrafish forebrain regions Dm and Dl (corresponding to basolateral amygdala and hippocampus respectively) and sensory inputs from visual and olfactory systems are the major drivers of spontaneous Hb activity. Our results suggested that these limbic and sensory inputs are integrated in Hb in a non-linear fashion and can regulate sensory representations in Hb. We propose that Hb lies in the heart of a brain wide network and act as “a hub” or “a switchboard”, which can regulate or gate the communication of sensory systems and limbic forebrain areas with the monoaminergic brainstem nuclei that control animal behaviors.

Disclosures: E. Yaksi: None. **E. Bartoszek:** None. **S. Kumar Jetti:** None. **S. Fore:** None.

Poster

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Topic: G.03. Emotion

Support: NIH R01MH097320

Title: Time course of emotional processing: A support vector machine classification study

Authors: *K. BO[1], A. TROGNETRPUNVA[1], Y. LIU[3], A. KEIL[2], M. DING[1];
[1]Dept. of Biomed. Engin., [2]The Dept. of Psychology and the NIMH center for Emotion and Attention, Univ. of Florida, Gainesville, FL; [3]Univ. of California, Davis, Davis, CA

Abstract: Emotional perception involves reciprocal interactions between subcortical and cortical structures. Functional magnetic resonance imaging (fMRI) is the dominant technique for identifying these structures in humans. Owing to the limited temporal resolution of fMRI, the detailed temporal dynamics of emotional processing remains to be further elucidated. We recorded electroencephalogram (EEG) in healthy controls and electrocorticogram (ECOG) in epilepsy patients during passive viewing of emotion pictures. 40 pleasant (erotica, romantic courtship, sport scenes), 40 neutral/calm (house hold scenes, people), and 40 unpleasant (mutilation, human violence, attacking animals) pictures, selected from the International Affective Picture System (IAPS), was presented in random order. Each picture was shown for 1000ms. The inter-trial interval (ITI) varied from 6000 to 9000ms. Applying a support vector machine (SVM) classifier to single-trial EEG and ECOG data we report the following findings. For EEG, the classification accuracy for pleasant versus neutral became significantly above chance level at around 220ms post stimulus onset, and reached a peak at around 480 ms. For unpleasant versus neutral comparison, the classification accuracy became significantly above chance level at around 300ms, and reached a peak at around 520 ms. The main contributing electrodes to the classification accuracy were located in occipital, left temporal and right frontal areas. For ECOG similar time courses were identified. Sites in broadly distributed brain areas contributed to the classification accuracy. Keywords: emotion; single-trial EEG; Ecog; support vector machine

Disclosures: **K. Bo:** None. **A. Trognetrpunva:** None. **Y. Liu:** None. **A. Keil:** None. **M. Ding:** None.

Poster

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Topic: G.03. Emotion

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ERC Advanced

Swiss National Foundation

Title: Neural ensemble dynamics underlying a long-term associative memory

Authors: ***B. F. GREWE**[1], J. GRÜNDEMANN[2], J. D. MARSHALL[3], J. LECOQ[4], L. J. KITCH[3], J. LI[3], F. GRENIER[2], A. LÜTHI[2], M. J. SCHNITZER[3];

[1]ETH Zurich, Zuerich, Switzerland; [2]Friedrich Miescher Inst., Basel, Switzerland; [3]Stanford Univ., Stanford, CA; [4]Allen Brain Inst., Seattle, WA

Abstract: The brain's ability to relate different events and stimuli is vital to the formation of associative memories. Extensive past research has examined molecular, synaptic and cellular level substrates of associative memory, but it remains unclear how large neural ensembles encode an associative memory. Using auditory fear conditioning, an established form of associative learning, we studied how neural ensembles in the mouse basal and lateral amygdala (BLA) represent conditioned and unconditioned stimuli (CS and US) and their learned association. To do this we monitored simultaneously the somatic calcium dynamics of ~150-200 individual BLA neurons per mouse using a miniature, head-mounted fluorescence microscope [Ghosh *et al.*,*Nature Methods* (2011)]. This approach allowed us to track the cells' evoked responses to the different stimuli throughout a six-day fear conditioning paradigm. Unforeseen from prior work that supported a simple synaptic Hebbian model in which a set of BLA 'fear neurons' encode the memory through potentiation of their CS-evoked responses, the ensemble codes revealed a combination of up- and down-regulation of individual cells' CS-evoked responses, which were equally important for storing the learned ensemble CS-US association. As mice learned to express conditioned behavioral responses, the ensemble neural representations of the CS and US gained in similarity. Throughout learning, the strength of the ensemble-encoded CS-US association was predictive of each mouse's behavioral performance. These findings extend the synaptic Hebbian model of associative learning to the neural ensemble level model in which activation of the US-representation provides a signal to supervise the transformation of the CS-representation. Overall, our results reveal the basic information processing steps by which BLA neural ensembles reliably encode a long-term associative memory and may generalize to other brain areas and forms of associative learning. Further, our approach to monitoring large populations of amygdala neurons opens the door to time-lapse imaging studies of neural coding in a brain structure that likely plays a central role in several widespread psychiatric disorders. As discussed, we should not write about extinction given that at the behavioral level there was no extinction, not even partial extinction.

Disclosures: **B.F. Grewe:** None. **J. Gründemann:** None. **J.D. Marshall:** None. **J. Lecoq:** None. **L.J. Kitch:** None. **J. Li:** None. **F. Grenier:** None. **A. Lüthi:** None. **M.J. Schnitzer:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Mark J. Schnitzer is a co-founder of and the chief scientist of Inscopix Inc., the company that manufacturers the integrated microscope..

Poster

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Program#/Poster#: 733.07/EEE3

Topic: G.03. Emotion

Support: NIMH IRP

Title: Amygdala preferentially projects to face selective areas in the monkey

Authors: *A. MESSINGER[1], J. M. SEIDLITZ[2], C. SPONHEIM[1], R. B. H. TOOTELL[3], L. G. UNGERLEIDER[1];
[1]Lab. of Brain and Cognition, NIMH, Bethesda, MD; [2]Brain Mapping Unit, Univ. of Cambridge, Cambridge, United Kingdom; [3]Ctr. for Biomed. Imaging, Massachusetts Gen. Hosp., Charlestown, MA

Abstract: In both humans and monkeys, emotional faces evoke greater functional MRI (fMRI) responses than neutral faces in the amygdala and in face-selective cortical areas (Pessoa et al. 2002; Hadj-Bouziane et al., 2008). Amygdala lesions eliminate this emotional modulation and impair the discrimination of facial emotions (Hadj-Bouziane et al., 2012; Adolphs et al. 1994). Thus, the correct interpretation of emotional facial expressions relies on amygdala feedback to face processing areas.
To map these feedback projections *in vivo*, we delivered electrical stimulation to specific amygdala nuclei in two rhesus monkeys and simultaneously monitored activity at the site of stimulation and in anatomically connected areas with fMRI (Tolias et al., 2005). Stimulation in the lateral nucleus of the amygdala (7 sites), a structure with primarily afferent projections, resulted in activation of the amygdala and the ipsilateral rhinal cortex, temporal pole, rostrotemporal auditory cortex, and insula. Stimulation in the ventral portion of the basal nucleus (4 sites) produced a similar pattern of activation. In contrast, sites in the dorsal portion of the basal nucleus (8 sites), a structure with primarily efferent projections, generated widespread bilateral activations that included much of the ventral visual stream, auditory cortex, insula, cingulate cortex, orbitofrontal cortex, lateral frontal cortex, and hippocampus. These activations reflect feedback projections from the basal nucleus to these areas, several of which have no reciprocal projections to the amygdala.
Stimulating the dorsal basal nucleus significantly activated both face- and object-selective regions (i.e., face and object patches) in the ventral visual stream, which were mapped in a separate visual fMRI localizer experiment. However, the stimulation-induced signal changes in the face patches were at least 40% greater than those in the object patches for both the stimulated and contralateral hemisphere in each animal. Thus, the amygdala exerted significantly greater influence on activity in ventral visual areas involved in face processing than in areas that process

inanimate objects (t-test, $p<0.01$ in each hemisphere). This stronger coupling with face patches was observed for all but one of the 8 stimulation sites in the dorsal basal nucleus. We conclude that cortical face processing can be particularly modulated by feedback signals from the amygdala, which likely convey the emotional state expressed by others and keep the viewer attuned to the socially salient features of the face.

Disclosures: A. Messinger: None. **J.M. Seidlitz:** None. **C. Sponheim:** None. **R.B.H. Tootell:** None. **L.G. Ungerleider:** None.

Poster

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Support: DFIPDF 2015 IITK 1953

Title: Effect of contextual word picture pairing on psychophysiological response

Authors: *M. ASTHANA, B. BHUSHAN;
Dept. of Humanities and Social Sci., Indian Inst. of Technol. Kanpur, Kanpur, India

Abstract: Several studies have shown that the presence of emotional stimuli alters brain circuitry. Neuroimaging evidences had affirmed the impaired brain circuitry in patients with *PTSD* during the processing of emotionally valenced word pair. However, it is unclear if processing of emotional valence of word-picture pairs affects the psychophysiological state of healthy individuals or not. In an on-going study we are investigating the alteration in psychophysiological response as a direct effect of emotional valence of the stimuli by comparing the relationship between word-picture association by means of subjective ratings and physiological responses. This study might be able to bring an insight on contextual relevance of word-picture pairing. With this study one might expect that if both word and picture are neutral then a slight alteration in the physiological responses is possible. However, if the word is neutral while the picture is emotional then one might expect a sudden and heightened change in physiological responses compared to the control condition (neutral word-picture pairing). The findings may contribute to the therapeutic approach to pathological learning and memory such as PTSD. Keywords: word, picture, physiological response, context, emotions

Disclosures: M. Asthana: None. **B. Bhushan:** None.

Poster

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Topic: G.03. Emotion

Title: Prepulse inhibition of a fear conditioned startle probe

Authors: *E. BJØRKEDAL[1], O. ÅSLI[2];
[1]Univ. of Tromsø, Tromsø, Norway; [2]Psychology, UiT Arctic Univ. of Norway, Tromsø, Norway

Abstract: Emotionally significant stimuli show enhanced neural responses early in sensory processing. This allows them to compete more effectively for processing resources and emotionally significant task irrelevant stimuli can interfere with task performance and capture attention. Prepulse inhibition (PPI) is the phenomenon where a weaker pre-stimulus inhibits the reaction to a later stronger startle-eliciting stimulus. The inhibition functions as a protection of the processing of the prepulse and is thought of as a measure of an automatic pre-attentive process. In the present experiment, the startle-eliciting stimulus was given enhanced emotional significance through fear conditioning in order to create a competition between the inhibition induced by the prepulse and the emotional salience of the startle-eliciting stimulus. If PPI is reduced in this scenario it would be evidence that emotionally salient stimuli interfere with a pre-attentive process at a short timescale. If PPI is unaffected, it would be evidence that the interference of emotionally salient stimuli are reduced by a prepulse. In a pilot study, the group receiving paired presentations of the pulse and the electrical stimulation had significantly smaller PPI after conditioning compared to before. Pilot data and data from main study will be presented.

Disclosures: E. Bjørkedal: None. **O. Åsli:** None.

Poster

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Topic: G.03. Emotion

Support: LSC Grant to AS & IK

DFG Grant SCHA1848/1-1

Title: Impacts of motivational, associated, and inherent emotional valence on face processing: evidence from event-related brain potentials (ERPs)

Authors: *A. SCHACHT[1,2], A.-M. GRIMM[1], W. HAMMERSCHMIDT[1,2], R. JACOB[1], I. KAGAN[3,2];
[1]Univ. of Goettingen, Goettingen, Germany; [2]Leibniz-ScienceCampus Primate Cognition, Goettingen, Germany; [3]German Primate Ctr., Goettingen, Germany

Abstract: Many studies indicate a tight relationship between cognition, emotion and motivation. Emotional as well as motivational factors have been demonstrated to modulate the processing of sensory stimuli at various processing stages, ranging from initial perception to elaborate stimulus evaluation. There is growing evidence that stimuli of emotional and motivational valence are preferentially processed, which is reflected not only on a behavioral but also on a neural level. Recently, it has been proposed that this processing advantage is not limited to stimuli with emotional content - inherently neutral stimuli also have been shown to acquire increased salience through motivational context and learning mechanisms. In the present study, we aimed at disentangling impacts of motivational, associated, and inherent emotional salience on face perception by means of event-related brain potentials (ERPs). During a learning session, participants (N=36) performed a prime-target face-matching task while emotionally neutral target faces were associated with monetary gain, loss or zero outcome, contingent on the matching task performance. Reward conditions were indicated by cues prior to the subliminally presented prime faces. On the following day, participants performed the same task, without monetary feedback, on the previously seen faces and, in addition, on unfamiliar faces showing happy, angry, or neutral expressions. Motivational, associated, and inherent salience types modulated morphologically similar ERP components starting around 200 ms after target onset, i.e. the Early Posterior Negativity (EPN) and the Late Positive Complex (LPC). In the learning session, both positive (gain) and negative (loss) motivational valence similarly boosted EPN and LPC, indicating enhanced attention to and elaborate processing of target faces with incentive value. In the test session, ERPs to associated negative salience were of reduced amplitudes during the delayed testing, whereas ERPs to unfamiliar faces indicated a processing bias towards angry faces. Together, our findings demonstrate qualitatively differential impacts of motivational, associated and inherent emotional valence on face perception.

Disclosures: **A. Schacht:** None. **A. Grimm:** None. **W. Hammerschmidt:** None. **R. Jacob:** None. **I. Kagan:** None.

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Topic: G.03. Emotion

Support: DFG - German Reserach Foundation

BMBF - German Federal Ministry of Education and Research EMOIO(16SVZ146)

Title: Frontal theta asymmetry and heart rate differentiate between emotional states in infants

Authors: *E. BOLINGER[1], D. HETTICH[1,2], K. ZINKE[1], V. KOCK[1], H.-V. NGO[1], T. MATUZ[1], J. BORN[1], N. BIRBAUMER[1,3];
[1]Inst. for Med. Psychology and Behavioural Neurobio., [2]Wilhelm-Schickard-Institute for Computer Sci., Univ. of Tübingen, Tuebingen, Germany; [3]Wyss Ctr. of Bio and Neuroengineering, Geneva, Switzerland

Abstract: Emotions dominate behavior. They are genetically predetermined and likely linked to specific brain modes of information processing. Here, we attempt to differentiate basic emotions by examining not only behavioral expression, but also central nervous system EEG activity and heart rate during emotional states in 4 to 6 month old infants. This age group provides a unique condition where positive and negative emotions are robustly expressed but where other cognitive faculties (i.e. language) that could mask emotions, are not yet highly developed. In this experiment, a social interaction paradigm was used to elicit the infant's emotional response in 5 naturalistic conditions, which were designed to evoke either positive or negative emotions. We hypothesized that behavioral differences in positive, neutral, and negative emotional states (which were identified by facial, vocal, and motor behavioral criteria) would lead to distinct changes in EEG rhythms and heart rate. An analysis of brain region (frontal, central, and parietal) and EEG power in the delta, slow theta (3-5 Hz), fast theta (5.5-7 Hz), alpha, and beta bands showed that emotional state had widespread influences on cortical activity, where negative states specifically were associated with high power across most EEG bands. Indeed, only frontal activity significantly differentiated between all three emotional states, with fast theta playing an integral role by discriminating between neutral and positive emotional states, such that positive emotion was accompanied by greater fast theta power. Based on these results, and because previous research suggests theta is involved in emotional processing, frontal asymmetry within the slow and fast theta bands was investigated. Interestingly, frontal asymmetry in these bands together differentiated between negative, neutral and positive states. While negative and positive states were associated with relatively more slow and fast theta power in the frontal left hemisphere, a neutral emotional state was related to greater fast theta power in the right hemisphere. Heart rate, on the other hand, was higher during negative emotional states but did

not differ between neutral and positive states. We conclude that positive and negative emotions manifest in robust changes in EEG theta power and hemispheric asymmetry, together with heart rate changes, already very early in life. Emotions appear to be genuinely connected to specific brain modes of information processing.

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Topic: G.03. Emotion

Support: RSF Grant 15-11-30014

Title: Using brain imaging techniques for validation of a biologically-inspired-cognitive-architecture model of human cognition

Authors: *A. V. SAMSONOVICH[1,2], V. L. USHAKOV[2,3];
[1]Krasnow Inst. Adv Study, George Mason Univ., Fairfax, VA; [2]Dept. of Cybernetics, Natl. Res. Nuclear Univ. MEPhI, Moscow, Russian Federation; [3]Natl. Res. Ctr. Kurchatov Inst., Moscow, Russian Federation

Abstract: The aim of this study is to develop an approach to evaluation of a biologically inspired, causal model of cognition that exposes the functional requirements for achieving emotional intelligence and makes testable predictions for neurophysiological measures. A theory of how concepts are represented in the human brain should specify (a) the structure and semantics of concept representations in the brain, and (b) types, formats and specific patterns of neuronal activity instantiating these representations. The key to a biologically-informed human brain model begins with the mapping of (a) to (b): in our case, of the emotional Biologically Inspired Cognitive Architecture (eBICA: Samsonovich, BICA, 2013) to informative features and characteristics of brain activity. The eBICA model is based on three main extensions of the standard building blocks of a cognitive architecture: a moral schema, an emotional state, and an emotional appraisal that is attributed to every cognitive representation in this model and determines dynamics of learning and decision making. The general cognitive cycle of eBICA includes perception, cognition, decision making and learning. The values of emotional appraisals and emotional states are sampled from the weak cognitive map (Samsonovich and Ascoli, 2010), which uses an abstract vector space to represent semantic relations among mental states, schemas

and their instances. This model is tentatively mapped onto the human brain, which allows us to test assumptions and predictions of the model; specifically, mechanisms of emotional thinking underlying behavior generation. The eBICA model can be validated based on this approach via comparison of the computational model behavior, the human participant behavior, and the localized non-invasive brain imaging data. The latter include fMRI, EEG, eye-tracking, ECG, EMG, and other psychophysiological measures. Methods involve the dynamic connectivity calculation and the use of regressors based on EEG and eye-tracking data. In an fMRI study, effective connectivity can be measured using Structural Equation Modeling, Granger Causality Analysis, Transfer Entropy and Dynamic Causal Modeling. The recording and analysis of eye movements provides access to the rapid unconcscious information processing. This approach has been applied to fMRI data collected in our study, representing human brain activity during cognitive tasks modulated by emotional state of the subject.

Disclosures: A.V. Samsonovich: None. **V.L. Ushakov:** None.

Poster

733. Emotion: Information Processing

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 733.13/EEE9

Topic: G.03. Emotion

Title: Difference in emotion information processing between individuals with high or low degrees of alexithymia

Authors: *K. GARCIA[1], K. JOHNS[2], E. DUBON[2], S.-M. KANG[2];
[1]Psychology, California State University, Northridge, North Hollywood, CA; [2]California State University, Northridge, Northridge, CA

Abstract: Alexithymia has been defined as the difficulty or inability of distinguishing, identifying, or expressing emotions (Kooiman, Spinhoven & Trijsburg, 2002). Previous research has demonstrated that individuals with high degrees of alexithymia (HDA) show an early processing deficit compared to individuals with low degrees of alexithymia (LDA) by focusing on P100 and P300 peaks in Event-Related Potential (ERP) patterns (Pollatos & Gramann, 2011). However, few attempts have been made to explore differences in the amplitude of N250 between individuals with high or low degrees of alexithymia, although past literature has shown that N250 is closely related to facial affect processing (Nasr & Esteky, 2009). The main purpose of the current study was to further explore differences in Event-Related Potential (ERP) patterns between individuals with HDA or LDA by focusing on the N250 peak. In this study, a total of 15 participants (6 low and 9 high) were selected based on their performance on the Toronto

Alexithymia Scale (Bagby et al., 1994) from the initial pool of 683 college students. In an individual session, a total of 11 electrodes were manually placed on a participant's head (PZ, CZ, and FZ) and face using the 10-20 international system. Each participant was instructed to identify emotion expressions shown on a computer screen either in the half or whole face condition. A total of 42 faces taken from the NimStim Face Stimulus Set (Tottenham et al., 2002) were used. The faces expressing six basic emotions (happy, fear, anger, disgust, surprise, sad) were presented either in the whole or half face condition. In the half condition, either the top or bottom half of the face was shown. The results of the current study showed that there was a significant 3-way interaction effect among face (whole vs. half) x condition (HDA vs. LDA) x location (PZ, CZ, and FZ) on the amplitudes of N250, F (2, 14) = 3.91, p = .045. This significant effect means that individuals with HDA (M = -5.68, SE = 3.52) tended to put more cognitive effort at the FZ site to configure emotion expressions from the half faces compared to the individuals with LDA (M = -1.37, SE = 3.94). This trend implies that the individuals with HDA need more later cognitive processes to configure emotion expressions, especially when facial information is limited. *Keywords: alexithymia, ERP, HDA/LDA, TAS-20*

Disclosures: **K. Garcia:** None. **K. Johns:** None. **E. Dubon:** None. **S. Kang:** None.

Poster

733. Emotion: Information Processing

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 733.14/EEE10

Topic: G.03. Emotion

Support: NWO

Title: Seeing an angry face impairs learning the value of information in an uncertain world

Authors: ***P. PIRAY**, V. LY, K. ROELOFS, R. COOLS, I. TONI;
Donders Inst., Nijmegen, Netherlands

Abstract: Recent theories suggest that emotions influence decision making by modulating the ability to learn the value of information in uncertain environments. However, the computational and neural mechanisms by which emotions modulate learning the value of information are still unclear. Here, we exploit recent advances in computational modeling to fill this gap. Human participants (N=44) underwent functional magnetic resonance imaging while performing a task requiring them to learn contingencies between face cues and financial outcomes. The emotional content of the face cues and the rate of change of the contingencies were independently manipulated. Participants processed emotional contents of cues and distinguished between

stochastic and systematic changes in task contingencies, as quantified with a Bayesian hierarchical learning model. Seeing angry faces made participants less sensitive to the causal statistics of changes in the environment, reducing their estimates of volatility. Neural signals associated with volatility-learning, in the right lateral prefrontal cortex (LPFC), were also disrupted. In contrast, seeing angry faces increased neural signals tracking volatility in the anterior cingulate cortex (ACC) and its volatility-dependent connectivity with the striatum. The change in ACC volatility-tracking induced by seeing angry faces was negatively correlated, across individuals, with the disruption in behavioral estimates of volatility. This observation suggests that ACC might partially compensate for that disruption in volatility tracking. Consistent with the opposite contributions of LPFC and ACC to volatility processing, volatility-tracking signal in ACC and volatility-learning signal in LPFC changed in opposite directions as a function of anger-induced changes in behavioral performance. These complementary and hierarchically-organized neurocomputational effects indicate that anger perception influences human decision-making by modulating higher-order computations related to learning value of information in uncertain environments. This study opens the way to formally characterize how psychiatric alterations of emotional processing change human decision making.

Disclosures: **P. Piray:** None. **V. Ly:** None. **K. Roelofs:** None. **R. Cools:** None. **I. Toni:** None.

Poster

733. Emotion: Information Processing

Location: Halls B-H

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Topic: G.03. Emotion

Support: National Institute of Mental Health 5SC2MH087466-02

MARC/MBRS Program

Title: P300 differences in emotion recognition between deaf signers and hearing non-signers

Authors: ***K. W. JOHNS, JR**, K. GARCIA, E. DUBON, S.-M. KANG;
Psychology, CSUN, Lancaster, CA

Abstract: It has been speculated that hearing impairment and early adoption of American Sign Language (ASL) should affect the development of basic mechanisms of face perception among deaf signers (Letourneau & Mitchell, 2011). The lack of auditory information may make deaf signers rely unduly on visual cues in order to recognize faces and emotional states. In addition to this, deaf signers should constantly pay attention to facial expressions (e.g., changes in eyebrow

or mouth shapes) because ASL employs a number of facial expressions as crucial linguistic components of communication (Letourneau, Maslin 2013). Due to this unique behavioral practice among deaf signers, it has been suspected that deaf signers might process facial information somewhat differently compared to normal hearing nonsigners. The current study was designed to further explore the findings from Letourneau and Mitchell's study (2011) using ERPs, focusing on the P300 component. The main purpose of the current study was to explore the differences in latencies and amplitudes of P300 between deaf signers and hearing non-signers, while they engaged in an emotion recognition test. Two emotion recognition tasks were administered to 14 deaf and 27 normally hearing college students, one with 42 full faces and another with 42 half faces, while their ERPs were recorded. The correct responses of the emotion recognition test were also recorded.
The results of the current study showed that there was a significant main effect of face on amplitude, F (1, 39) = 13.23, $p < .001$. For both deaf and hearing group, they significantly had greater amplitudes in the half face condition in comparison to the whole face condition. There was also a significant main effect of hearing condition on latency, F (1, 39) = 5.39, p =.026. The deaf group had a longer latency than the hearing group. The longer latency of P300 among deaf participants implied that they had more difficulty to configure emotional expressions than the other group. This difficulty was reflected in their performance on the emotion recognition test. The results of the analyses on the correct responses of half faces showed that deaf participants tended to have more difficulties to configure facial expressions than the normally hearing participants, t (39) = 1.71, p = .095. The significance and implication of the findings were discussed in details, focusing on the impact of ASL and deafness on emotion information processing. This research was supported by a grant from the National Institute of Mental Health 5SC2MH087466-02 and MARC/MBRS Program.

Disclosures: **K.W. Johns:** None. **K. Garcia:** None. **E. Dubon:** None. **S. Kang:** A. Employment/Salary (full or part-time): PI.

Poster

733. Emotion: Information Processing

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 733.16/EEE12

Topic: G.03. Emotion

Support: Grants-in-Aid for Scientific Research from the Japan Society for the Promotion of Science No. 25290014

Title: Non-edited isoform of 5-HT_{2C} receptor affects NPY expression in the nucleus accumbens and behavioral despair in mice

Authors: *M. TANAKA[1], M. AOKI[2], A. TSUJIMURA[3], K. TAGUCHI[1], Y. WATANABE[3]; [1]Anat. and Neurobio., [2]Dent. Med., [3]Basic Geriatrics, Kyoto Prefectural Univ. Med., Kyoto, Japan

Abstract: Serotonin 2C receptors (5-HT_{2C}R) are widely expressed in the central nervous system, and are associated with various neural functions such as emotion, food intake, dependence and stress response. 5-HT_{2C}R mRNA is known to undergo adenosine-to-inosine RNA editing at five sites within its coding sequence, resulting in expression of 24 different isoforms. Several edited isoforms show reduced activity. This fact suggests that RNA editing can modulate serotonergic systems in the brain with causative relevance to neuropsychiatric disorders. Transgenic mice solely expressing the non-edited 5-HT_{2C}R INI-isoform (INI-mice) or the fully edited VGV-isoform (VGV-mice) exhibit various phenotypes including metabolic abnormalities, aggressive behavior, anxiety-like behavior, and depression-like behavior. Here, we examined the behavioral phenotype and molecular changes of INI-mice on a C57BL/6J background. INI-mice showed an enhanced behavioral despair in the forced swimming test (FST), elevated sensitivity to the tricyclic antidepressant, desipramine, and significantly decreased 5-HT in the nucleus accumbens (NAc), amygdala, and striatum. They also showed reduced expression of neuropeptide Y (NPY) mRNA in the NAc and its expression level in wild type (WT)-mice was decreased after 5-HT_{2C}R selective agonist, WAY-629. However immobility time in FST was not significantly changed after microinjection of NPY or NPY-Y1 receptor agonist, [Leu31, Pro34]-NPY, were bilaterally microinjected into the NAc of WT- or INI-mice. Next we examined FST after increased NPY mRNA by stereotactic injection of adeno-associated virus encoding NPY into the NAc, and demonstrated that accumbal NPY overexpression decreased immobility time. Our results suggest that accumbal NPY expression may be regulated by 5-HT_{2C}R RNA editing, and its impairment may be linked to mood disorders.

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Poster

733. Emotion: Information Processing

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Program#/Poster#: 733.17/EEE13

Topic: G.03. Emotion

Support: JSPS KAKENHI Grant Number 15K00210

Title: Respiratory synchronized activations of olfactory processing in the limbic system

Authors: *Y. MASAOKA[1], M. YOSHIDA[2], N. KOIWA[3], K. WATANABE[1], A. YOSHIKAWA[1], M. IDA[4], I. HOMMA[5], M. IZUMIZAKI[1];
[1]Dept. of Physiol., Showa Univ. Sch. of Med., Tokyo, Japan; [2]Dept. of Ophthalmology, Jikei Med. Univ., Tokyo, Japan; [3]Univ. of Human Arts and Sci., Human Arts and Sciences Research Center, Japan; [4]Dept. of Radiology, Stroke Ctr., Ebara Tokyo Hosp., Tokyo, Japan; [5]Tokyo Ariake Univ. of Med. and Hlth. Sci., Tokyo, Japan

Abstract: Olfaction is dependent on respiration for the delivery of odorants to the nasal cavity. Taking advantage of the time-locked nature of inspiration and olfactory processing, electroencephalogram dipole modeling (EEG/DT) has previously been used to identify a cascade of inspiration-triggered neural activity moving from primary limbic olfactory regions to frontal cortical areas during odor perception. In this study, we combined functional magnetic resonance imaging (fMRI) and EEG in order to re-examine the neural cascade model of human olfactory processing higher spatial and temporal resolution. Twenty right-handed healthy volunteers participated in this study. This study was approved by the ethics committee of Showa University School of Medicine and all participants provided informed consent. The fMRI study was divided into 2 sessions/runs: (i) periods of pleasant odor interleaved with unscented air, and (ii) periods of no-odor interleaved with unscented air. Each session comprised 5 unscented and 5 scented blocks or no-odor blocks, with a duration of 30s each. Subjects were instructed to breathe normally throughout the experiment. Statistical analysis of fMRI data was performed using statistical parametric mapping (SPM12) software, and Drifter toolbox installed in SPM8 was used to remove physiological noises of respiration and cardiac signals from fMRI-BOLD signals. Inspiration-related fMRI activations during olfaction stimuli were observed in the primary olfactory areas including piriform cortex, amygdala, entorhinal cortex and hippocampus. Brain activation identified by both modalities converged within same olfaction-related areas, and EEG/DT was additionally provided that the primary olfactory regions activated occurring approximately 50ms-100ms post-inspiration, and the orbitofrontal cortex that were activated from approximately 150ms to 300ms after inspiration onset. These results provide a partial validation of the spatial profile of the olfactory cascade identified by EEG source modeling, and inform novel future directions in the investigation of human olfaction.

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Poster

734. Biomarkers for Animal Models of Depression

Location: Halls B-H

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Program#/Poster#: 734.01/EEE14

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: NCN Grant UMO-2012/07/B/NZ7/01164

Title: Stress resilient rats show reciprocal changes in the expression of miRNAs associated with regulation of serotonin transporter level in mesocortical circuit

Authors: *D. ZURAWEK, M. KUSMIDER, A. FARON-GORECKA, M. KOLASA, J. SOLICH, P. PABIAN, P. GRUCA, M. DUBIEL, K. SZAFRAN-PILCH, M. PAPP, M. DZIEDZICKA-WASYLEWSKA;
Inst. of Pharmacol. PAS, Krakow, Poland

Abstract: Stress perturbs psychological and physiological balance of an individual. Exposure to prolonged stress can lead to depression. Nevertheless, some individuals are more resilient to stress than the others. In our present study we used Chronic Mild Stress (CMS) paradigm - well characterized animal model of depression. Chronic exposure of rats to unpredictable mild stressors caused behavioral deficits (anhedonia) manifested by decreased consumption of a palatable 1% sucrose solution (significant effect of stress $F_{2,129}=72.42$; $p<0.0001$, time $F_{2,129}=14.57$; $p<0.0001$ and stress x time $F_{4,129}=26.57$; $p<0.0001$ vs control). Moreover, CMS generated a proportion of animals demonstrating resilience manifested as not altered consumption of sucrose solution despite being stressed. Thus, CMS model mimic natural variety in behavioral response to stress observed among people because not all subjects experiencing chronic stress develop depression. Recently, epigenetic regulation of a gene expression associated with microRNA (miRNA) is considered as an important factor modulating biochemical response to stress. In our present study, based on our previous work and literature survey, we investigated changes in the expression level of 7 miRNAs (i.e. miR-18a-5p, miR-34a-5p, miR-135a-5p, miR-195-5p, miR-320-3p, miR-674-3p, miR-872-5p) in mesocortical circuit - crucially involved in stress response in order to find differences between stress susceptible (anhedonic) and resilient phenotype. Our bioinformatic analysis using MirWalk2.0 software showed that all miRNAs of interest have a potential to regulate serotonin transporter (SERT) expression. RT-qPCR analysis revealed that exposure to 2 weeks of CMS caused global increase in the expression of measured miRNAs in ventral tegmental area (VTA) of stressed rats followed by parallel decrease in miRNA expression in prefrontal cortex (PCx). This effect was more profound in resilient animals than in anhedonic ones. Next, we examined binding of [^{3}H]paroxetine to SERT to check whether global changes in the expression of miRNAs potentially regulating SERT may be associated with altered SERT expression in vivo. After 2

weeks of CMS we observed decreased level of SERT in VTA of resilient rats ($F_{2,11}$= 9.15, p< 0.01 vs control and anhedonic). Our findings show that mesocortical circuit is involved in the response to a challenging conditions and this phenomenon is more efficient in resilient animals. This response is shown at the level of miRNA expression what, in turn, influenced downstream biochemical pathway associated with SERT expression. This work was supported by the NCN grant UMO-2012/07/B/NZ7/01164, Poland.

Disclosures: **D. Zurawek:** None. **M. Kusmider:** None. **A. Faron-Gorecka:** None. **M. Kolasa:** None. **J. Solich:** None. **P. Pabian:** None. **P. Gruca:** None. **M. Dubiel:** None. **K. Szafran-Pilch:** None. **M. Papp:** None. **M. Dziedzicka-Wasylewska:** None.

Poster

734. Biomarkers for Animal Models of Depression

Location: Halls B-H

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Program#/Poster#: 734.02/FFF1

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: JSPS KAKENHI 25430077

Title: Contrasting expression patterns of inflammation-related genes in mouse models of depression and psychosis

Authors: *H. KOSHIMIZU, H. HAGIHARA, T. MIYAKAWA;
ICMS, Fujita Hlth. Univ., Toyoake, Japan

Abstract: Previously, we showed the existence of pseudo-immature brain cell states in the dentate gyrus (DG) of mouse models of psychiatric disorders, including schizophrenia and bipolar disorder. It was also demonstrated that some brain cells can undergo rejuvenation in response to the external stimulation, such as treatment with antidepressant (fluoxetine; FLX), pilocarpine-induced seizure, and physiological stimulation. Pseudo-immature brain cell states are often associated with inflammation. Recently, via bioinformatics analysis, we indicated transcriptomic “hypermaturity” in the DG of mice overexpressing the glucocorticoid receptor (GRov mice), which show increased depression-like and anxiety-like behaviors and are considered potential animal models for mood disorders, and the hippocampus of the glutamate dehydrogenase 1 (Glud1) transgenic mice and mice treated with PF-04447943, a selective phosphodiesterase-9 (PDE9) inhibitor. However, it is largely unknown whether there is any common molecular basis for “hypermature” and pseudo-immature brains. Here, we compared genome-wide gene expressions in the DG of GRov mice with those in inflammation by using a bioinformatics tool, NextBio. The gene expression patterns in the DG of GRov mice showed

statistically significant similarity to those in the inflammation-associated events, such as poly(I:C) infection and colitis. Among genes that were upregulated or downregulated in "hypermature" brains, there were significant enrichments in signal pathways related to inflammation and immune reactions. Both these enrichments were also observed for pseudo-immature brains. Of the inflammation and immune-related genes in the pseudo-immature brains, the number of upregulated genes was significantly greater than that of downregulated genes. In contrast, in the "hypermature" brains, downregulations were dominant to upregulations in the inflammation and immune-related genes. These observations indicate that inflammation is commonly involved in both pseudo-immature and "hypermature" brains, and each of them may represent unique inflammation-related events.

Disclosures: H. Koshimizu: None. **H. Hagihara:** None. **T. Miyakawa:** None.

Poster

734. Biomarkers for Animal Models of Depression

Location: Halls B-H

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Program#/Poster#: 734.03/FFF2

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: NIMH MH077159

Title: Role of dendritic-targeting bdnf transcripts on depression- and stress-associated phenotype

Authors: *H. OH[1], D. LEWIS[2], S. WATKINS[3], S. PIANTADOSI[2], E. SIBILLE[1];
[1]Dept. of Psychiatry, CAMH, Toronto, ON, Canada; [2]Dept. of Psychiatry, [3]Dept. of Cell Biol. and Physiol., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: *Introduction.* A parallel downregulation of brain-derived neurotrophic factor (BDNF) and somatostatin (SST), a marker of inhibitory γ-amino-butyric acid (GABA) interneurons which target pyramidal cell dendrites, has been reported in multiple brain areas of subjects with brain disorders, including major depressive disorder (MDD). Our previous study revealed that BDNF transcripts with long 3' untranslated region (3' UTR), which have been reported to migrate to distal dendrites, are selectively downregulated and highly correlated with markers of dendritic-targeting interneurons including SST, in the prefrontal cortex of MDD subjects and of chronically stressed mice. The aim of this study is to investigate if altered dendritic BDNF is causal to the MDD- and stress-associated molecular, structural and behavioral changes. *Methods.* Using small hairpin RNA (shRNA) targeting long 3' UTR of BDNF mRNA, we knocked down dendritic BDNF transcripts in primary mouse cortical neurons and measured total length, number of segments of dendrites. AAV expressing BDNF long 3' UTR targeting shRNA or scrambled

shRNA were injected into the mPFC of male C57BL/6J mice and changes of MDD-associated synaptic gene expression and depressive-/anxiety-like behavior were analyzed. *Results.* Suppression of long 3' UTR (+) BDNF mRNA decreased the length of dendrite of primary cortical neurons without changing number of dendritic segments. BDNF long 3' UTR as well as total BDNF level were significantly reduced in post-surgery week 4, and SST level was changed along with BDNF mRNAs in week 6. Gene manipulation exacerbated depressive- and anxiety-like behavior in mice in response to stress. *Conclusions.* Knockdown of dendritic BDNF recapitulated MDD- and chronic stress-associated phenotypes, namely impaired integrity of distal dendrites, increased behavioral emotionality and reduced SST gene expression. These findings provide evidence for a novel pathogenic mechanism in MDD linking local neurotrophic support, pyramidal cell structure and dendritic inhibition.

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Poster

734. Biomarkers for Animal Models of Depression

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Program#/Poster#: 734.04/FFF3

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: NARSAD

Campbell Family Mental Health Research Institution Grants

Title: Dynamic molecular changes over the course of chronic stress exposure in mice and correlation with behavioral phenotype

Authors: *T. D. PREVOT, K. MISQUITTA, H. OH, E. SIBILLE, M. BANASR;
Campbell Family Mental Hlth. Res. Inst., CAMH, Toronto, ON, Canada

Abstract: Major depressive disorder (MDD) is a complex pathology where multiple cell-specific pathways interact to produce heterogeneous phenotypes. Growing evidence shows that MDD is associated with molecular changes within GABA cells, astrocytes and synapses in the prefrontal cortex (PFC). Similar changes are reported in stress-based rodent models of depression. GABA cells and astrocytes are closely interacting to regulate synaptic neurotransmission. However the sequence of changes affecting these compartments is poorly understood in the context of MDD.

Here, we aim to identify dynamic changes in cell type markers during chronic stress exposure and their correlations with the onset of depressive-like behaviors. Using the chronic restraint stress (CRS) paradigm (1h restraint stress, 2 x day) in C57/BL6 male and female mice, we determined CRS effects on behavior at different time points (1 to 5 weeks) and on the expression of markers for GABA cells, astrocytes and synapses. We assessed the progressive CRS effects on anxiety-like behavior (acute response to a 1h spotlight stressor in home cage-like setting), anhedonia-like deficits and coat deterioration. Characterization of the cellular alterations induced by CRS was performed using western blot and qPCR on PFC samples. Results show that CRS induced time-dependent deficits in anxiety-like and anhedonia-like behavior. Indeed, CRS-exposed mice exhibit an increase in latency to normalise to control level after the light challenge. This residual avoidance behavior was significant after 1, 2 and 5 weeks of CRS. Moreover, CRS induced a time dependent increase in anhedonia-like behavior assessed by a reduction of sucrose consumption significant from the 3rd week of CRS. At the cellular level, western blot analysis confirmed the reductions in GABAergic (GAD67), astrocytic (GFAP, glutamine synthetase (GS)) and synaptic (PSD-95, Syn1) markers following 5 weeks of CRS. Preliminary analyses suggest that GFAP and GS expression profile correlates with anhedonia-like deficits trajectory, while GAD67 expression follows the increased avoidance phenotype in response to acute stress challenge. In addition, synaptic markers progressively decreased over the course of the CRS exposure. Ongoing experiments will confirm these dynamic changes using qPCR and extend this study to GABA neuron subtypes and other astrocytic and synaptic markers. Here we confirm that chronic stress is a multiphasic process engaging short-term changes that progressively lead to a pathological state. Our results suggest that different cellular systems are altered at those stages, in parallel to different behavioral outcomes.

Disclosures: T.D. Prevot: None. **K. Misquitta:** None. **H. Oh:** None. **E. Sibille:** None. **M. Banasr:** None.

Poster

734. Biomarkers for Animal Models of Depression

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Program#/Poster#: 734.05/FFF4

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: The MEXT, Japan

The MAFF, Japan

SIP, The Cabinet Office, Japan

Title: Behavioral and metabolic characterization in subchronic social defeat model of mice

Authors: *A. TOYODA[1,2], T. GOTO[1], H. OTABI[1], S. TOMONAGA[3], D. KOHARI[1,2], T. OKAYAMA[1,2];
[1]Agr., Ibaraki Univ., Ibaraki, Japan; [2]Tokyo Univ. of Agr. and Technol., Fuchu, Japan; [3]Agr., Kyoto Univ., Kyoto, Japan

Abstract: Previously, we developed a depression model of mice using the paradigm of subchronic mild social defeat stress (sCSDS) (Goto *et al*., Behav Brain Res 2014). sCSDS mice (C57BL/6J, male) showed increased body weight gain, polydipsia, and social avoidance. Also, sCSDS mice severely delayed nest building process (Otabi *et al*., Behav Process 2016). The social avoidance behavior in sCSDS mice were affected by the diet purity, namely sCSDS mice fed non-purified diet (MF, Oriental Yeast, Japan) showed more resilience compared to sCSDS mice fed purified diet (AIN-93G, Oriental Yeast) (Goto *et al*., Nutr Neurosci *in press*). To assess these underlying mechanisms, metabolites in plasma, liver, and cecum digesta of sCSDS mice were analyzed by metabolomics using a gas chromatography-mass spectrometry system under two different feeding conditions, purified pellet diet (AIN-93G) and non-purified pellet diet (MF). Four test groups were set as 'sCSDS + AIN-93G diet', 'sCSDS + MF diet', 'control (without sCSDS) + AIN-93G diet', and 'control + MF diet'. The sCSDS mice were produced as previously. Two-way ANOVA were used to compare the factors ‘stress’, ‘food’, and ‘stress × food’. To control the *p*-value for multiple comparisons, the false discovery rate was determined. The significance threshold was set at $Q < 0.1$. Metabolome analysis revealed that the diet effect on metabolites is larger than the stress effect. Namely, 22, 27, and 31 of metabolites in plasma, liver, and cecum, respectively, were significantly changed by diet, while 8 and 5 of metabolites were significantly changed by sCSDS in plasma and liver, respectively. Also, in the interaction of stress and diet, 5 and 1 of metabolites were significantly changed in plasma and cecum, respectively. Possibly, these metabolites have important roles in resiliency and vulnerability to psychosocial stress, which will be assessed in the future study.

Disclosures: A. Toyoda: None. **T. Goto:** None. **H. Otabi:** None. **S. Tomonaga:** None. **D. Kohari:** None. **T. Okayama:** None.

Poster

734. Biomarkers for Animal Models of Depression

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: Délégation Générale à l’Armement (DGA)

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Institut National de la Santé et de la Recherche Médicale (INSERM)

Université Pierre et Marie Curie (UPMC)

Title: Electroencephalographic biomarkers for vulnerability to depression in the rat.

Authors: *D. CLAVERIE[1,2,3,4], C. BECKER[2,3,4,5], A. GHESTEM[6,7], M. COUTAN[8], F. CAMUS[2,3,4], C. BERNARD[6,7], J.-J. BENOLIEL[2,3,4,9], F. CANINI[8,10];
[1]IRBA, Bretigny Sur Orge FRANCE, France; [2]Sorbonne Universités, UPMC Univ. Paris 06, UMCR18, Neurosciences Paris-Seine, site Pitié-Salpêtrière, Paris, France; [3]INSERM, U 1130, Paris, France; [4]CNRS, UMR 8246, Paris, France; [5]Univ. Paris Descartes, Sorbonne Paris Cité, Faculté de Médecine, Paris, France; [6]Univ. Aix Marseille, INS, Marseille, France; [7]Inserm, UMR_S 1106, Marseille, France; [8]Dept. Neurosciences & Contraintes Opérationnelles, Inst. de Recherche Biomédicale des Armées (IRBA), Brétigny-sur-Orge, France; [9]Service de Biochimie Endocrinienne et Oncologique, Hôpital de la Pitié-Salpêtrière, Paris, France; [10]Ecole du Val de Grâce, Paris, France

Abstract: After exposure to a highly stressful situation, some individuals become at high-risk to develop depression later in life. A low serum Brain Derived Neurotrophic Factor (sBDNF) level one month after stress exposure signs this state of vulnerability in both rodents and Human. We used electroencephalograms (EEG) recorded during active waking periods to test for the presence of predictive EEG biomarkers of vulnerability to depression before a highly stressful situation in rats. EEG and sBDNF were analyzed before (Baseline), 5 (Post-stress) and 31 days (Recovery) after social defeat (SD) in 43 rats. We show that future vulnerable animals could readily be identified before SD by higher high theta and alpha spectral relative powers and lower beta-2 main peak frequency. These differences were maintained after SD. Low beta-2 main peak frequency was predictive of future vulnerability. Such biomarker could easily be used to identify human populations at-risk for depression.

Disclosures: D. Claverie: None. **C. Becker:** None. **A. Ghestem:** None. **M. Coutan:** None. **F. Camus:** None. **C. Bernard:** None. **J. Benoliel:** None. **F. Canini:** None.

Poster

734. Biomarkers for Animal Models of Depression

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 734.07/FFF6

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: NARSAD

Campbell Family Mental Health Research Institute

NIH

CCDR University of Toronto (KAM)

Banting Postdoctoral Fellowship (YSN)

Title: Unpredictable chronic mild stress induces correlated depressive-like behavioral and MRI brain volume changes in mice

Authors: *K. A. MISQUITTA[1,2], Y. S. NIKOLOVA[1], B. ROCCO[1], J. ELLEGOOD[4], J. LERCH[4], M. BANASR[1], E. SIBILLE[1,2,3];
[1]Campbell Family Mental Hlth. Res. Inst., Ctr. for Addiction and Mental Hlth., Toronto, ON, Canada; [2]Pharmacol. and Toxicology, [3]Psychiatry, Univ. of Toronoto, Toronto, ON, Canada; [4]Mouse Imaging Ctr., The Hosp. for Sick Children, Toronto, ON, Canada

Abstract: Major depressive disorder (MDD) is a severe mental illness characterized by low mood, anhedonia, and often associated with heightened anxiety. Human magnetic resonance imaging (MRI) studies have described structural alterations in key brain regions involved in MDD including amygdala, hippocampus, and prefrontal cortex. Parallel studies in stress-based rodent models have provided insight into the putative molecular mechanisms underlying these morphological and behavioral changes; however, a clear link between these features has yet to be determined. To help bridge this gap, we used MRI to assess brain morphology in the unpredictable chronic mild stress (UCMS) mouse model of depression, after behavioral characterization. Using Noldus phenotypers apparatus, we monitored the weekly baseline behavior (time spent in drinking, food, shelter zones) of 8-12 week-old BALB/c mice (n=12/group) to obtain longitudinal measures over the course of the UCMS protocol. At the end of the UCMS protocol we administered acute behavioral tests including the elevated plus maze, open field, novelty suppressed feeding, cookie test, forced swim test and sucrose consumption. A principal component analysis (PCA) was used to obtain a summary measure of UCMS-induced heightened emotionality across tests. Finally, brains were perfused with contrasting agent and submitted to 7T MRI to assess volumetric MRI changes of twenty-seven brain regions associated with human MDD. Our longitudinal assessment of behavior demonstrated that UCMS exposure induced a progressive increase in the time spent hiding in shelter in disfavor of other zones within the arena. This increase in hiding behavior in the UCMS group was further exacerbated by an acute stress challenge (spotlight over the food zone). UCMS-exposed animals showed higher scores on the top behavioral principal component and clear separation from control mice ($p = 8 \times 10\text{-}7$) capturing 23.5% of variance across tests. Finally, we demonstrated an increase in volume of the amygdala, hippocampus, frontal association, prelimbic and cingulate cortices of UCMS animals, relative to the controls. These morphological changes were positively correlated with the top principal component of the behavioral data. Experiment in progress will determine the cellular correlates associated with UCMS-induced increases in volume of the amygdala. Altogether this work will identify cellular and morphological substrates underlying depressive-

like behaviors and will provide a translational perspective on the biological mechanisms involved in human MDD.

Disclosures: K.A. Misquitta: None. **Y.S. Nikolova:** None. **B. Rocco:** None. **J. Ellegood:** None. **J. Lerch:** None. **M. Banasr:** None. **E. Sibille:** None.

Poster

734. Biomarkers for Animal Models of Depression

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 734.08/FFF7

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Title: Differential gene expression in blood cells and brain regions of resilient and vulnerable animals after acute, uncontrollable and severe stress

Authors: X. YAO[1], J. SHOBLOCK[1], *G. CHEN[2];
[1]Janssen R & D, San Diego, CA; [2]Neurosci. Drug Discovery, Janssen R&D, LLC, Janssen Pharm. Comp. of JNJ, San Diego, CA

Abstract: Stress disorders such as depression and Post Traumatic Stress Disorder (PTSD) are outcomes of the interplay between the genetic polymorphisms and early life insults derived resilient and vulnerable factors and the ongoing psychological and physical stress; however, the molecular identities of the resilient and vulnerable factors are still largely unknown. Similar to the chronic social defeat stress paradigm, the learned helplessness model offers an opportunity to explore the identities of potential resilient and vulnerable factors under the acute, uncontrollable and severe stress. After unavoidable foot shocks delivered in random intervals, a portion of animals develops deficits in the active avoidance test, which has been considered as a measure of behavior despair. Samples of peripheral blood mononuclear cell (PBMC), prefrontal cortex, hippocampus, amygdala and cerebellum were collected and then submitted to gene expression analysis using RNA sequencing platform. The expression of numerous genes was up or down-regulated in all tissue types after the stress. More genes were up- or down regulated in the amygdala than other brain regions, a finding consistent with the known function of amygdala as the stress relay center. More genes in PBMC than in brain regions were differentially changed in resilient and vulnerable animals. The bioinformatics revealed that the large portion of the differentially regulated genes belongs to the neuroendocrine and immune response systems or involved in cell structure and cellular resilience. This data provides starting information to further explore the genes and gene networks in model animals and human subjects in order to develop novel treatments, prevention measures, and predictive and diagnostic biomarkers for depression and PTSD.

Disclosures: **X. Yao:** A. Employment/Salary (full or part-time): Janssen R & D / Full. **J. Shoblock:** A. Employment/Salary (full or part-time): Janssen R & D / Full. **G. Chen:** A. Employment/Salary (full or part-time): Janssen R & D/ Full.

Poster

734. Biomarkers for Animal Models of Depression

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 734.09/FFF8

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: RIKEN Junior Research Associate fellowship

MEXT/JSPS KAKENHI

Grant-in-Aid from the Japanese Ministry of Health and Labor

Title: Search for plasma biomarkers of depression by metabolome analysis in an animal model and clinical samples

Authors: *Y. KAGEYAMA;
Osaka City Univ., Osaka-Shi, Japan

Abstract: There is an urgent need for biomarkers of major depressive disorder to optimize its treatment. Although various candidate biomarkers were reported, no one has been established. The purpose of the present study was to search for novel biomarkers using human plasma samples and depression mouse model (mutant *Polg* 1 transgenic mouse) that spontaneously showed depressive episode-like behavioral changes. We measured plasma metabolites, by capillary electrophoresis time-of-flight mass spectrometry, in the mutant mice (10 euthymic state and 10 depressive episode-like state) and human participants matched age and sex with no medication (8 major depressive disorder and 19 healthy controls). The animal experimental protocols were approved by the Wako Animal Experiment Committee, RIKEN. The human study was approved by the ethics committees of Hannan Hospital, Osaka City University, RIKEN, and National Center of Neurology and Psychiatry and conducted in accordance with the Declaration of Helsinki. Plasma concentration of betaine ($p=0.024$), glutamic acid ($p=0.040$), and creatine ($p=0.049$) were higher in the mouse model of depression-like state than euthymic state. Plasma concentration of cis-aconitic acid was higher in patients with major depressive disorder than healthy controls ($p=0.039$). Betaine, glutamic acid and creatine did not show significant change between patients with major depressive disorder and healthy controls. These three metabolites were not a marker of major depressive disorder but warrants further regression

analysis and investigation in a larger number of samples to draw a conclusion whether there is a state-dependent alteration. We also measured plasma metabolites in patients with bipolar disorder and schizophrenia and will discuss the results in the poster.

Disclosures: Y. Kageyama: None.

Poster

734. Biomarkers for Animal Models of Depression

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 734.10/FFF9

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: Biohaven Pharmaceuticals

Title: Riluzole prevents the onset of anxiety-, anhedonia-, and helplessness-like deficits in two rodent models of stress-related illness.

Authors: *C. J. FEE[1,2], E. SIBILLE[1,3,2], R. M. BERMAN[4], V. CORIC[4], G. SANACORA[5,4], M. BANASR[1,4];
[1]Campbell Family Mental Hlth. Res. Inst., Ctr. For Addiction and Mental Hlth., Toronto, ON, Canada; [2]Pharmacol. and Toxicology, [3]Psychiatry, Univ. of Toronto, Toronto, ON, Canada; [4]BioHaven Pharmaceuticals, New Haven, CT; [5]Psychiatry, Yale Univ. Sch. of Med., New Haven, CT

Abstract: Anxiety, anhedonia, and helplessness are common symptoms associated with major depressive disorder (MDD), schizophrenia (SCZ), and post-traumatic stress disorder (PTSD). Dysregulated glutamate function is thought to underlie these symptoms across disorders. Riluzole decreases glutamate transmission as an enhancer of glutamate uptake and a sodium channel blocker. Previous studies suggest riluzole possesses antidepressant-like and anxiolytic properties. However, therapeutic indication in other psychiatric mood disorders has not been established. Here, using two stress-based rodent paradigms that model the negative symptoms of SCZ and PTSD-like deficits, we examined riluzole's ability to prevent the development of these behavioral deficits. We first sought to determine the behavioral efficacy of riluzole in mice exposed to unpredictable chronic mild stress (UCMS) using tests measuring anhedonia and anxiety. We then examine riluzole effects on helplessness-like deficits using a learned helplessness (LH)/active avoidance (AA) paradigm.
Adult C57BL/6J mice underwent 5 weeks of randomized mild stressors (2-4/day). Riluzole (60 µg/mL) was administered in sweetened drinking water (saccharin 0.1%) throughout UCMS exposure. Elevated plus maze (EPM), open field test (OFT), and novelty suppressed feeding

(NSF) were performed to measure anxiety-like behavior. Sucrose consumption (SC) measured anhedonia-like behavior only, while novelty induced hyperphagia (NIH) measured both anxiety- and anhedonia-like behavior. Behavioral changes were assessed by Z-scoring across tests measuring similar outcomes. In a separate cohort, riluzole was administered for 14 days prior exposure to LH. Mice received 60 inescapable footshocks (0.35 mA) and then tested in AA (30 trials). Escape failures was recorded as an index of helplessness-like deficits. The composite Z-anxiety score, calculated for EPM, OFT, NSF, and NIH, demonstrated significantly heightened emotionality following UCMS that was prevented by riluzole. This effect was driven by an attenuation of UCMS-induced effects in NIH and EPM. Riluzole treatment also prevented UCMS-induced anhedonia z-scored from NIH and SC. The anti-anhedonia effect of riluzole was highly significant in both tests. In the LH paradigm, riluzole significantly attenuated helplessness-like behavior, as indexed by a reduction in escape failures. This data supports the utility of riluzole as an indication for treating, and possibly preventing, anhedonia and anxiety symptoms associated with stress-related disorders, including MDD, SCZ, and PTSD.

Disclosures: **C.J. Fee:** 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; BioHaven Pharmaceuticals. **E. Sibille:** None. **R.M. Berman:** A. Employment/Salary (full or part-time): BioHaven Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); shares in BioHaven Pharmaceuticals Holding Company. **V. Coric:** A. Employment/Salary (full or part-time): BioHaven Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); shares in BioHaven Pharmaceuticals Holding Company, co-inventor on a patent “Glutamate agents in the treatment of mental disorders” Patent number: 8778979. **G. Sanacora:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; AstraZeneca, Bristol-Myers Squibb, Eli Lilly & Co., Johnson & Johnson, Hoffman La-Roche, Merck & Co., Naurex, Servier. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Sanofi-Aventis. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); shares in BioHaven Pharmaceuticals Holding Company, co-inventor on a patent “Glutamate agents in the treatment of mental disorders” Patent number: 8778979. F. Consulting Fees (e.g., advisory boards); Allergan, Alkermes, AstraZeneca, BioHaven Pharmaceuticals, Hoffman La-Roche, Janssen, Merck, Naurex, Servier Pharmaceuticals, Taisho Pharmaceuticals, Teva, VistaGen Therapeutics. **M. Banasr:** 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; BioHaven Pharmaceuticals.

Poster

734. Biomarkers for Animal Models of Depression

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 734.11/FFF10

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: Young Invest

Title: Pre-existing differences in white matter diffusivity correlate with behavioral response to acute social defeat in mice

Authors: *Y. GROSSMAN[1], D. DUMITRIU[2];
[1]Icahn Sch. of Med. At Mount Sinai, New York, NY; [2]Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: Depression is a neuropsychological disorder that affects millions of people. There are currently no predictors for susceptibility to depression and a large proportion of afflicted individuals are resistant to available treatments. The ability to predict selective psychosocial vulnerability and resilience to stress holds great promise in preventing this debilitating disorder. Social defeat (SD) is a highly validated mouse model of depression. Previously, we had demonstrated using a model of acute social defeat (ASD), that animals exhibiting socially avoidant (susceptible) and resilient behavioral responses possessed differences in functional responses to acute social stress. Here, we probe the neuroarchitecture that could provide a foundation for these variances observed in functional connectivity. We performed diffusion-weighted imaging (DWI) one week prior to ASD then correlated the diffusivity of various brain regions with behavioral response to ASD. We found a positive correlation between anisotropy and resilience to ASD, indicating that differences in white matter connectivity may be a pre-existing condition that contributes to the differences in functional connectivity that we had previously reported.

Disclosures: Y. Grossman: None. **D. Dumitriu:** None.

Poster

734. Biomarkers for Animal Models of Depression

Location: Halls B-H

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: AIHS #200700595

NSERC

CIHR #102652

Title: Ancestral stress induces sexually dimorphic effects in depressive-like behaviours of aging rats

Authors: *M. AMBESKOVIC[1], O. BABENKO[1], E. A. FALKENBERG[1], Y. ILNYTSKYY[2], I. KOVALCHUK[2], G. A. S. METZ[1];
[1]Canadian Ctr. for Behavioural Neurosci., Univ. of Lethbridge, Lethbrigde, AB, Canada; [2]Dept. of Biol. Sci., Univ. of Lethbridge, Lethbridge, AB, Canada

Abstract: INTRODUCTION: Exposure to stress in early life represents a powerful impact on fetal brain development and hypothalamic-pituitary-adrenal (HPA) axis activity. Moreover, adverse early life experiences may also increase the susceptibility to affective disorders, such as depression, in adulthood. Our previous studies showed that recurrent prenatal stress across generations has cumulative effects on brain plasticity and generates new behavioural traits. Here we proposed that recurrent prenatal stress also compromises mental health trajectories in older age.
This study investigated in rats the effect of: (1) aging on depression-like behaviours, stress response and epigenetic regulation by microRNAs; (2) cumulative ancestral prenatal stress (APS) on aging in terms of depression-like behaviour in males versus females.
METHODS: Male and female F4 generation offspring were derived from a rat lineage in which their ancestral mothers (F0-F3) were stressed during pregnancy. A non-stress lineage served as control. Depression-like behaviours were assessed at the age of 6 (young), 12 (middle aged) and 18 (aged) months using a forced swim task. Behavioural outcomes were related to plasma corticosterone levels, and microRNA expression in the prefrontal cortex.
RESULTS: Aging raised the incidence of depression-like behaviours in both males and females regardless of APS. APS however further exacerbated depression-like behaviour in males along with decreased cortical expression of miR-124, a recognized biomarker and therapeutic target in depression. In contrast, APS reduced age-associated depression-like symptoms in females and increased cortical miR-124 expression. In addition, aging and stress synergistically disturbed HPA axis activation and accelerated an age-associated decline in affective state in males.

CONCLUSION: Ancestral programming by stress may represent a significant determinant of lifetime mental health trajectories, a major contributor to sexually dimorphic phenotype, and a risk factor for common age-related diseases through altered epigenetic regulation. MiR-124 may be used as predictive biomarkers of age-related diseases in males.

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Poster

735. Treatment and Drug Discovery: Depression

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 735.01/FFF12

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Title: P11, an essential cell-specific molecular determinant for stress-induced depression

Authors: *J.-S. SEO[1], J. WEI[2], L. QIN[2], Z. YAN[2], P. GREENGARD[1];
[1]The Rockefeller Univ., New York, NY; [2]Dept. of Physiol. and Biophysics, Sch. of Med. and Biomed. Sci., State Univ. of New York, Buffalo, NY

Abstract: Chronic stress plays a crucial role in the development of psychiatric diseases, such as anxiety and depression. Dysfunction of the medial prefrontal cortex (mPFC) has been linked to the cognitive and emotional deficits induced by stress. However, little is known about the molecular and cellular determinants in mPFC leading to stress-associated mental disorders. Here we show that chronic restraint stress induces the selective loss of p11 (also known as annexin II light chain, S100A10), a multifunctional protein which binds to 5-HT receptors, in layer II/III neurons of the prelimbic cortex (PrL), as well as depression-like behaviors, both of which are reversed by selective serotonin reuptake inhibitors (SSRIs) and the tricyclic class of antidepressant (TCA) agents. In layer II/III of the PrL, p11 is highly concentrated in dopamine D2 receptor-expressing ($D2^+$) glutamatergic neurons. Viral expression of p11 in $D2^+$ PrL neurons alleviates the depression-like behaviors exhibited by genetically manipulated mice with $D2^+$ neuron-specific or global deletion of p11. In stressed animals, overexpression of p11 in $D2^+$ PrL neurons rescues depression-like behaviors by restoring glutamatergic transmission. Our results have identified p11 as a key molecule in a specific cell type that regulates stress-induced depression, providing a framework for the development of new strategies to treat stress-associated mental illnesses.

Disclosures: J. Seo: None. **J. Wei:** None. **L. Qin:** None. **Z. Yan:** None. **P. Greengard:** None.

Poster

735. Treatment and Drug Discovery: Depression

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NIH U24 DK097154 West Coast Comprehensive Metabolomics Center

Title: Gene deficiency and pharmacological inhibition of soluble epoxide hydrolase confers resilience to repeated social defeat stress: role of BDNF-TrkB signaling

Authors: *Q. REN[1], M. MA[1], T. ISHIMA[1], C. MORISSEAU[2], J. YANG[2], K. M. WAGNER[2], J.-C. ZHANG[1], C. YANG[1], W. YAO[1], C. DONG[1], M. HAN[1], B. D. HAMMOCK[2], K. HASHIMOTO[1];
[1]Chiba Univ. Ctr. Forensic Mental Hlth., Chiba, Japan; [2]Dept. of Entomology and Nematology, Univ. of California Davis Comprehensive Cancer Ctr., Davis, CA

Abstract: Depression is a severe and chronic psychiatric disease, affecting 350 million subjects worldwide. Although multiple antidepressants have been used in the treatment of depressive symptoms, their beneficial effects are limited. The soluble epoxide hydrolase (sEH) plays a key role in the inflammation which is involved in depression. Thus, we examined here the role of sEH in depression. In both inflammation and social defeat stress models of depression, a potent sEH inhibitor TPPU displayed rapid antidepressant effects. Expression of sEH protein in the brain from chronically stressed (susceptible) mice was higher than of control mice. Furthermore, expression of sEH protein in postmortem brain samples of patients with psychiatric diseases, including depression, bipolar disorder, and schizophrenia, was higher than controls. This suggests that increased sEH levels might be involved in the pathogenesis of certain psychiatric diseases. In support of this hypothesis, pretreatment with TPPU prevented the onset of depression-like behaviors after inflammation or repeated social defeat stress. Moreover, sEH knock-out (KO) mice did not show depression-like behavior after repeated social defeat stress, suggesting stress resilience. The sEH KO mice showed increased brain-derived neurotrophic factor (BDNF) and phosphorylation of its receptor TrkB in the prefrontal cortex (PFC),

hippocampus, but not nucleus accumbens, suggesting that increased BDNF-TrkB signaling in the PFC and hippocampus confer stress resilience. All these findings suggest that sEH plays a key role in the pathophysiology of depression, and that epoxy fatty acids, their mimics as well as sEH inhibitors could be potential therapeutic or prophylactic drugs for depression.

Disclosures: **Q. Ren:** None. **M. Ma:** None. **T. Ishima:** None. **C. Morisseau:** None. **J. Yang:** None. **K.M. Wagner:** None. **J. Zhang:** None. **C. Yang:** None. **W. Yao:** None. **C. Dong:** None. **M. Han:** None. **B.D. Hammock:** None. **K. Hashimoto:** None.

Poster

735. Treatment and Drug Discovery: Depression

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: NICSM Program Grant

Department of Education and Learning

Title: Investigation of the oral microbiome for candidate markers of depression.

Authors: ***C. R. LAPSLEY**[1], M. MCLAFFERTY[2], A. MCDOWELL[1], A. J. BJOURSON[1], S. M. O'NEILL[2], E. K. MURRAY[1];
[1]Northern Ireland Ctr. for Stratified Med., Ulster Univ., Derry, United Kingdom; [2]Psychology, Ulster Univ., Magee Campus, Derry, United Kingdom

Abstract: Depression is a complex disorder with multiple symptoms, including a persistent low mood, anhedonia and cognitive impairments, and is currently the third leading cause of global disability. The underlying pathophysiology of depression is poorly understood, and the overall diagnosis and selection of treatment course for depression is largely subjective, reliant on patient self-report and clinical judgment. There is therefore an urgent need for new biological markers of depression and treatment response. A growing body of evidence supports an important role for the microbiome in the aetiology of depression and other psychiatric disorders. While much interest is currently focused on the role of the microbiome-gut-brain axis in brain physiology and neurochemistry, the importance of the oral microbiome has received little attention. The aim of this study is to characterise the oral and dormant blood microbiome in adults with moderate and severe depression versus matched controls with no history of the disease. To achieve this, participants were asked to complete an online validated mental health survey and to provide a saliva sample. We identified 46 individuals who met the DSM-V criteria for severe depression

and 46 age and sex-matched controls with no history of depression. Bacterial DNA was extracted from the saliva samples and 16S rRNA surveys were conducted using next generation sequencing. Differences in the bacterial community composition of the oral microbiota between patients and controls were determined, and validated using qPCR. Charting the oral/ blood microbiomes in depressed patients could therefore provide new insights into the development of the condition, and the identification of novel diagnostic and therapeutic response biomarkers.

Disclosures: C.R. Lapsley: None. **M. McLafferty:** None. **A. McDowell:** None. **A.J. Bjourson:** None. **S.M. O'Neill:** None. **E.K. Murray:** None.

Poster

735. Treatment and Drug Discovery: Depression

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: NIH Grant GM100829

NIH Grant DA033877

NIH Grant GM083883

Title: Effects of repeated paroxetine and fluoxetine exposure on hippocampal BDNF functioning in adolescent rats

Authors: *J. M. DHARGALKAR, K. N. RUDBERG, A. SOLIS, Z. R. HARMONY, E. J. MACEDO, L. C. ROMERO, C. A. CRAWFORD;
Dept. of Psychology, California State Univ., San Bernardino, CA

Abstract: Selective serotonin reuptake inhibitors (SSRIs) are the most commonly prescribed class of antidepressant drugs, largely because of their effectiveness and favorable side-effect profiles. Unfortunately, the use of SSRIs in pediatric populations is limited due to reduced efficacy and their tendency to induce suicidal ideation in adolescents. Recently, we found that repeated treatment with fluoxetine and paroxetine caused increased anxiety-like behaviors in adolescent rats, as measured on the elevated plus maze and light/dark box. Moreover, adolescent rats did not show the adult-typical response of decreased serotonin utilization after repeated paroxetine treatment. The purpose of the present study was to determine if changes in brain derived neurotropic factor (BDNF) functioning are responsible for mediating these age-dependent behavioral and neurochemical effects. The rationale for this study was based on a growing body of evidence suggesting that the therapeutic effects of antidepressants are

dependent on BDNF-mediated increases in neurogenesis. To test our hypothesis, we measured the expression of BDNF and the BDNF receptor, TrkB, after repeated paroxetine and fluoxetine treatment. Male and female adolescent Sprague-Dawley rats (*n*=6-7) were injected with paroxetine (2.5 or 10 mg/kg), fluoxetine (5 or 10 mg/kg), or vehicle for 10 consecutive days starting on postnatal day (PD) 35. On PD 45, the hippocampus of each rat was removed and then assayed for BDNF and TrkB expression using western blotting. In both male and females rats, BDNF expression was decreased after fluoxetine (5 and 10 mg/kg) treatment. Paroxetine (10 mg/kg) also decreased BDNF levels, but only in male rats. In contrast, TrkB expression was increased after SSRI treatment; however, this increase was only significant for male rats treated with the high dose of fluoxetine (10 mg/kg). In summary, repeated treatment with the SSRIs paroxetine and fluoxetine led to decreased BDNF expression in adolescent rats. This reduction in BDNF levels may be responsible for the reduced efficacy of SSRIs during adolescence. Interestingly, paroxetine had a greater effect on the BDNF functioning of male rats than females.

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Poster

735. Treatment and Drug Discovery: Depression

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: Japanese Ministry of Education, Culture, Sports, Science and Technology, grant-in-aid No. 23591667

Hokkaido University Clark Memorial Foundation

Title: The role of medial prefrontal corticosterone and dopamine in the antidepressant-like effect of exercise

Authors: *S. NAKAGAWA[1,2], C. CHEN[2], Y. KITAICHI[2], Y. AN[2], M. KOGA[2], I. KUSUMI[2]; [2]Psychiatry, [1]Hokkaido Univ. Grad Sch. Med., Sapporo, Japan

Abstract: Despite the well-documented beneficial effect of exercise on stress coping and depression treatment, its underlying neurobiological mechanism remains unclear. This is further complicated by a 'side effect' of exercise: it increases basal glucocorticoid (CORT), the stress hormone, which has been shown to be a mediator linking stress to depressive disorders. Here we show that three weeks of voluntary wheel running reduced rats' immobility in the forced swim

test (FST), an antidepressant-like effect. Monitoring extracellular fluids in the medial prefrontal cortex PFC (mPFC) using microdialysis we found that, wheel running was associated with higher baseline CORT, but lower FST-responsive CORT. Further, wheel running resulted in a higher dopamine (DA) both at baseline and following FST. Interestingly, the antidepressant-like effect of wheel running was completely abolished by intra-mPFC pre-microinjection of a D2R (haloperidol) but not D1R (SCH23390) antagonist, at a dose that does not affect normal rats' performance in the FST. It suggests that exercise exerts antidepressant-like effect through upregulated DA and in a D2R dependent way in the mPFC. Importantly, the antidepressant-like effect of wheel running was also abolished by intra-mPFC pre-microinjection of a GR antagonist (RU486). Finally, intra-mPFC pre-microinjection of RU486 also downregulated the originally elevated basal and FST-responsive DA in the mPFC of exercise rats. These results suggest a causal pathway linking CORT, GR, DA, and D2R, to the antidepressant-like effect of exercise. In conclusion, exercise achieves antidepressant-like effect through the CORT-GR-DA-D2R pathway and that the increased basal CORT by exercise itself may be beneficial rather than detrimental. This research was supported by Japanese Ministry of Education, Culture, Sports, Science and Technology, grant-in-aid No. 23591667 (S.N.) and a grant from Hokkaido University Clark Memorial Foundation (C.C.).

Disclosures: **S. Nakagawa:** None. **C. Chen:** None. **Y. Kitaichi:** None. **Y. An:** None. **M. Koga:** None. **I. Kusumi:** None.

Poster

735. Treatment and Drug Discovery: Depression

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 735.06/FFF17

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Title: 3,5,6,7,8,3′,4′-heptamethoxyflavone, a citrus flavonoid, ameliorates depression-like behavior and brain-derived neurotrophic factor expression

Authors: ***A. SAWAMOTO**, S. OKUYAMA, K. YAMAMOTO, Y. AMAKURA, M. YOSHIMURA, M. NAKAJIMA, Y. FURUKAWA;
Col. of Pharmaceut. Sci., Matsuyama Univ., Matsuyama, Ehime, Japan

Abstract: The decreases in hippocampal BDNF levels are correlated with stress-induced depressive behavior and that antidepressant enhances the expression of BDNF. Our findings that 3,5,6,7,8,3′,4′-heptamethoxyflavone (HMF), a citrus flavonoid, has the potential for increasing the expression of the BDNF in the hippocampus of a transient global ischemia mouse model prompted us to investigate whether HMF has anti-depressive activity. A depression mice model

was developed through subcutaneous administration of corticosterone (20 mg/kg/day for 25 days), and HMF was simultaneously administered with corticosterone. As results, we observed that the HMF treatment ameliorated (1) corticosterone-induced body weight loss, (2) corticosterone-induced depression-like behavior, (3) corticosterone-induced reductions in BDNF production in the hippocampus, (4) corticosterone-induced reductions in neurogenesis in the dentate gyrus subgranular zone and (5) corticosterone-induced reductions in the expression levels of phosphorylated calcium-calmodulin-dependent protein kinase II and extracellular signal-regulated kinase1/2. These results provide that HMF has a possibility to prevent the onset of depression as an antidepressant by inducing the expression of BDNF.

Disclosures: **A. Sawamoto:** None. **S. Okuyama:** None. **K. Yamamoto:** None. **Y. Amakura:** None. **M. Yoshimura:** None. **M. Nakajima:** None. **Y. Furukawa:** None.

Poster

735. Treatment and Drug Discovery: Depression

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: NSF IOS-1355158

NIH-NIAAA pilot grant provided by the Integrated Neuroscience Initiative for Alcoholism

Department of Defense USAMRMC Award W81XWH-14-10061

Title: Rapid antidepressant spine formation is influenced by $GABA_B$ receptor activation of mTORC1

Authors: *C. F. HEANEY[1], E. R. WORKMAN[2], E. K. ERICSSON[2], K. F. RAAB-GRAHAM[1]; [1]Wake Forest Baptist Med. Hlth., Winston Salem, NC; [2]Univ. of Texas at Austin, Austin, TX

Abstract: Treatments for patients with major depressive disorder often take several weeks to become effective. Further, patients may not respond to the prescribed drug and thus begin a cycle of dose and drug changes. N-methyl-D-aspartate glutamate receptor (NMDAR) antagonists have emerged as rapid antidepressants, acting within hours of administration. What is more, these drugs are effective at treating patients who are treatment resistant. However, the cellular mechanisms associated with how these ligands produce their effects are unclear. NMDAR antagonists have been demonstrated to increase neurogenesis and activate the mammalian target of rapamycin (mTOR). Our lab has previously demonstrated that the administration of NMDAR

antagonists causes a functional shift in metabotropic gamma-aminobutyric acid (GABA) receptors ($GABA_BRs$). This shift decreases the coupling of $GABA_BRs$ to potassium channels and instead promotes coupling to calcium channels. Blocking $GABA_BRs$ reverses this effect. Here, we examined the interaction of rapid antidepressants and $GABA_BR$ ligands on synapse formation in order to further characterize this pathway. We demonstrate that these synaptic changes are likely newly formed synapses requiring protein synthesis. These data suggest a beneficial role of $GABA_BR$ signaling in rapid antidepressant mechanisms.

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Poster

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: NIH MH068542 (Hen)

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NIH DA022413 (Javitch)

Title: The antidepressant actions of tianeptine require the mu-opioid receptor

Authors: K. M. NAUTIYAL[1], B. A. SAMUELS[1], A. C. KRUEGEL[2], M. R. LEVINSTEIN[1], V. M. MAGLONG[1], M. M. GASSAWAY[2], M. A. ANSONOFF[4], J. E. PINTAR[4], B. L. KIEFFER[5], J. A. JAVITCH[1], D. SAMES[2], *R. HEN[3,6];
[1]Columbia Univ. and NYSPI, New York, NY; [2]Dept. of Chem. and NeuroTechnology Ctr., [3]Neurosci. and Psychiatry, Columbia Univ., New York, NY; [4]Dept. of Psychology, Behavioral & Systems Neurosci., Rutgers, The State Univ. of New Jersey-New Brunswick, Piscataway, NJ; [5]Douglas Institute, McGill, Montreal, QC, Canada; [6]NYSPI, New York, NY

Abstract: The majority of pharmacological agents used for the treatment of depression target the serotonin system. However, these drugs like selective serotonin reuptake inhibitors (SSRIs) are not ideal because only a fraction of patients achieve remission. New targets for antidepressant drugs are needed for this 'treatment resistant' population and additionally for patients who suffer from intolerable side-effects stemming from SSRI administration. The recent discovery that the effective antidepressant tianeptine (Stablon) is a full agonist at the mu-opioid receptor (MOR) has revealed a potential novel target for drug development. Our studies are aimed at

understanding the neural circuits through which tianeptine exerts its antidepressant effects. Using genetic and pharmacological models we tested whether the behavioral effects of tianeptine are mediated by MOR. We found that MOR knockout mice have no measured behavioral response to tianeptine. Additionally, the behavioral effects are also abolished by pretreatment with an MOR antagonist. Tissue-specific MOR knockout mice are now being tested to dissect the circuitry which underlies the opioid-dependent antidepressant action. Preliminary evidence suggests that the antidepressant phenotype is dissociable from the classic opiate phenotype including effects on activity, feeding, pain, and reward. Additionally, unlike other classic opiates, there seems to be no tolerance or withdrawal formed following chronic administration of tianeptine to mice. Taken together, these results point to a novel entry point to understand the neural circuits underlying depression, and a potential avenue for the development of a new class of antidepressant drugs.

Disclosures: **K.M. Nautiyal:** None. **B.A. Samuels:** None. **A.C. Kruegel:** None. **M.R. Levinstein:** None. **V.M. Maglong:** None. **M.M. Gassaway:** None. **M.A. Ansonoff:** None. **J.E. Pintar:** None. **B.L. Kieffer:** None. **J.A. Javitch:** None. **D. Sames:** None. **R. Hen:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Servier.

Poster

735. Treatment and Drug Discovery: Depression

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Program#/Poster#: 735.09/FFF20

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: HDRF MPPN8883

Title: Discovering common molecular signatures underlying antidepressant response

Authors: *E. CARAZO[1], C. ANACKER[1], B. A. SAMUELS[3], M. J. MEANEY[4], R. HEN[2]; [1]Integrative Neurosci., Columbia Univ., NEW YORK, NY; [2]Psychiatry, Neuroscience, and Pharmacol., Columbia Univ., New York, NY; [3]Psychology, Rutgers Univ., New Brunswick, NJ; [4]Ludmer Ctr. for Neuroinformatics and Mental Hlth., Douglas Mental Hlth. Univ. Institute, McGill Univ., Montreal, QC, Canada

Abstract: Anxiety and depressive disorders have a prevalence of 31% and 17% respectively in the United States population. Affecting over 40 million people, together they comprise the most common mental illnesses in the country. Despite the large number of patients suffering from anxiety and depressive disorders, there are limited treatment options. Antidepressants such as SSRIs are amongst the most common treatment options for depressive disorders. However,

despite being so widely used, only one-third of patients experience remission after up to four months of treatment with SSRIs, pointing to a clear need for a better understanding of the molecular and cellular mechanisms underlying SSRIs effects in order to develop better treatment options. On a neuroanatomical level, the dentate gyrus of the hippocampus has been shown to be a crucial mediator of antidepressant effects on behavior. Our aim is to study the neurobiological and genetic basis of antidepressant response and treatment resistance in order to identify novel molecular targets that mediate antidepressant treatment responses. We combined next-generation RNA sequencing and microarray data of fluoxetine treated mice, in order to identify molecular signaling programs that mediate the response to fluoxetine in the Dentate Gyrus. For this purpose we have used three complementary mouse models of chronic stress: social defeat, chronic corticosterone, and chronic oral gavage. All three models were similarly treated with fluoxetine for 28 Days, in order to elicit a common fluoxetine-induced genetic signature. Treatment effectiveness was determined in our models by segregating the fluoxetine responders and non-responders. The measure of responsiveness to fluoxetine was given by the levels of anxiety exhibited in behavioral tasks such as the social interaction test or novelty suppressed feeding after fluoxetine treatment. RNA from both dorsal and ventral dentate gyrus was extracted from the samples and processed by Microarray or RNA-sequencing. Our preliminary analysis point at a differential gene expression between dorsal and ventral dentate gyrus upon fluoxetine treatment. Furthermore, there are similarly regulated genes in fluoxetine responders across the datasets such as Npas4 and genes belonging to the activin/inhibin pathway, both of which have been implicated in antidepressant response. Ongoing efforts are aimed at identifying a common genetic signature that is specific to antidepressant responders and non-responders.

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Poster

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: SHRF-EG

NSERC-DG

Title: Altered membrane protein clustering in peripheral lymphocytes in an animal model of depression parallels protein clustering observed in patients with depression: a translational approach

Authors: R. ROMAY-TALLON[1], E. Y. FENTON[5], M. A. MITCHELL[2], L. E. KALYNCHUK[3], *H. J. CARUNCHO[4];
[1]Dept. of Med., [2]Psychology, [3]Col. of Med., [4]Col. of Pharm. and Nutr., Univ. of Saskatchewan, Saskatoon, SK, Canada; [5]Ctr. for Drug Res. and Develop., Vancouver, BC, Canada

Abstract: Membrane protein clustering (MPC) in peripheral lymphocytes is altered in depression and has been proposed as a putative biomarker of therapeutic efficacy in major depressive disorder (reviewed in Frontiers in Cellular Neuroscience, 2016,10:48). In this experiment, we analyzed the clustering of four transmembrane proteins in lymphocytes from rats repeatedly treated with corticosterone (CORT), a well-characterized animal model of depression. Male rats received 21 days of daily CORT injections (40 mg/kg) or vehicle injections. On day 22, rats were subjected to the forced swim test and blood samples were collected by cardiac puncture. Lymphocytes were extracted from the blood samples and processed for immunocytochemical analyses of the serotonin transporter (SERT), serotonin 2A receptor (5-HT2A), beta-2 adrenergic receptor (β-2AR), and the cellular prion protein (PrPc). CORT produced a depressive phenotype evidenced by significant increases in immobility behavior in the forced swim test. CORT also altered the pattern of clustering for all examined proteins. We found significant increases of 13%, 6% and 12% in the average size of SERT, 5-HT2A and PrPc clusters respectively, in the CORT rats compared to the vehicle rats. However, the β-2AR clusters were 7% smaller in the CORT rats. No changes were found in the number of SERT, 5-HT2A and β-2AR clusters per lymphocyte, but there was a 20% increase in the number of PrPc clusters in the CORT rats. We also found a positive correlation between the size of SERT, 5-HT2A and PrPc clusters and immobility behavior in the forced swim test. Importantly, the pattern of SERT and 5-HT2A protein clustering seen after CORT treatment is very similar to what we have previously observed in naïve depression patients. This suggests that parallel changes in protein clustering can be seen across a preclinical animal model of depression and the clinic, offering significant translational potential. In addition, our novel observation of altered β2AR and PrPc protein clustering in the animal model should be confirmed in a patient cohort. Overall, we believe that mapping changes in protein clustering in both patients and an animal model will better identify biomarkers for depression diagnosis and treatment responsivity.

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Poster

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: NIH Grant MH063266

Title: *In vivo* pharmacological testing of novel, short-acting kappa opioid receptor antagonists

Authors: S. PAGE[1], E. ROBERTS[2], H. ROSEN[2], D. PUTTICK[1], M. MAVRIKAKI[1], W. A. CARLEZON, Jr.[1], *E. H. CHARTOFF[3];
[1]Psychiatry, Harvard Med. Sch., Belmont, MA; [2]Chem., The Scripps Res. Inst., La Jolla, CA; [3]Psychiatry, Harvard Med. Sch. Dept. of Psychiatry, Belmont, MA

Abstract: Kappa opioid receptors (KOR) are expressed in brain areas implicated in motivation, emotion, and learning. Although selective KOR agonists share the antinociceptive effects of opiates, they tend to produce aversive- and stress-like effects in humans and laboratory animals. In contrast, selective KOR antagonists have antidepressant-like and anxiolytic-like effects, and can block drug-seeking behaviors in rodent models of addiction. These types of findings have stimulated interest in the therapeutic potential of KOR antagonists. However, prototypical KOR antagonists such as JDTic and norBNI have exceptionally long durations of action, complicating their use in clinical trials. Here we present initial pharmacological and behavioral data on a novel KOR antagonist, CYM 52220, synthesized at The Scripps Research Institute. CYM 52220 has high selectivity for KORs compared to other opioid receptors and favorable pharmacokinetic and distribution to brain (>4:1 brain-plasma ratio) profiles. Using the Tail Flick Assay (TFA), we performed dose-effect and time course experiments to quantify the ability of oral administration of CYM 52220 to block the antinociceptive effects of the KOR agonist U50,488 (30 mg/kg, IP). For comparison, we also tested the KOR antagonists JDTic and LY2456302, which have long and short durations of action, respectively. Consistent with previous reports, oral administration of JDTic (20 mg/kg; PO) blocked U50,488-induced increases in tail flick latency in the TFA for at least 24 hrs whereas the KOR antagonist effects of LY2456302 (0.9 mg/kg; PO) in the TFA were only observable for 1 hr after oral administration. CYM 52220 (0.0 – 30 mg/kg; PO) dose-dependently blocked U50,488-induced increases in tail flick latency when administered 2-hr prior to testing. An AD_{80} dose of CYM 52220 (6.0 mg/kg; PO) attenuated the analgesic effects of U50,488 for more than 2 hrs. In conclusion, CYM 52220 is a novel, potent, selective, and orally active KOR antagonist with a relatively short duration of action that may have therapeutic potential.

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Poster

735. Treatment and Drug Discovery: Depression

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 735.12/FFF23

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Title: The function of P-glycoprotein after chronic treatment with antidepressant drugs in the mice

Authors: *A. SHOHEI, K. OSADA, T. HAGA, T. WATANABE, H. KOCHA;
Neuropsychiatry, St.Marianna Univ. of Med., Kawasaki/Kanagawa, Japan

Abstract: P-glycoprotein (P-gp) is a 130-kDa adenosine triphosphate (ATP)-dependent drug transport protein that is abundantly distributed in the apical side of brain capillary endothelial cells forming the tight junctions of the blood-brain barrier (BBB). Utilizing ATP hydrolysis as an energy source, P-gp belongs to a large and growing group of transmembrane transporters, which are increasingly recognized as an important part of the blood-brain and blood-cerebrospinal fluid (CSF) barriers. P-gp is expressed by and was first discovered in multiple drug-resistant (MDR) cancer cells, but can also be found in normal tissue. P-gp has been demonstrated to influence the absorption, distribution, and elimination of many commonly used drugs. It has, furthermore, been shown that P-gp influences the distribution of drugs across the BBB. The location of P-gp at the BBB is of importance for the delivery of psychotropic drugs such as antidepressant and antipsychotic medications. Recently, it has also been demonstrated that the intracerebral concentrations of some psychotropic drugs and opioids can be up to several-fold higher in P-gp knock-out mice than in wild-type mice. Among these drugs are the antidepressants nortriptyline, escitalopram, sertraline, venlafaxine and fluvoxamine, the antipsychotic drugs olanzapine and risperidone, as well as the substrates for P-gp. But we do not know how the P-gp function after the chronic treatment with antidepressant drugs in the brain. Then we investigated which chronic treatment with the antidepressant drugs as the substrates for P-gp was changed the function of P-gp in the brain. C57BL/6N mice (weighing 20-25 g) were orally administrated of 10 mg/kg/day antidepressant drugs once daily for six weeks. To quantify the amount of mRNA in mice brain, we performed real-time PCR (7500 Fast Real-Time PCR System)by using TaqMan Fast Universal PCR Master Mix (life technologies). A PCR reaction mixture of 20µl containing 10µl of TaqMan Fast Universal PCR Master Mix, 9 µl of cDNA and 1 µl TaqMan Gene Expression Assays. We examined that the expression of RNA P-glycoprotein after chronic treatment with the antidepressant drugs and compared with before treatment.

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Poster

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: Academy of Finland (TR)

Title: Insights into the antidepressant mechanisms of (some) anesthetics and sedatives

Authors: *T. P. RANTAMAKI[1], N. MATSUI[2], S. KOHTALA[3], H.-K. WIGREN[3], T. PORKKA-HEISKANEN[3], A. KLEIN[4], W. THEILMANN[3];
[1]Univ. Helsinki, Helsinki, Finland; [2]Tokushima Bunri Univ., Tokushima, Japan; [3]Univ. of Helsinki, Helsinki, Finland; [4]Univ. of Copenhagen, Copenhagen, Denmark

Abstract: Post-ictal burst-suppressing electroencephalographic (EEG) activity has been proposed to underlie the neuroplastic and therapeutic effects of ECT (*electroconvulsive therapy)*. This hypothesis prompted researchers already decades ago to test and show that deep brief isoflurane anesthesia bring rapid antidepressant effects in some patients. The hypothesis was never tested experimentally however. Instead, some of the subsequent human trials produced conflicting results and thereby reduced the interest to further evaluate isoflurane as a substitute for ECT. We demonstrate here that burst-suppressing EEG is not prerequisite for the ability of isoflurane to regulate signaling responses implicated in the antidepressant actions of NMDA-receptor blocker ketamine: activation and inhibition of TrkB-mTor-p70S6k pathway and GSK3β, respectively. Indeed, and similarly with ketamine, already subanesthetic doses of isoflurane and *gamma*-hydroxybutyrate (GHB), another putative sedative antidepressant acting predominantly through NMDA/AMPA-independent pathways, regulate these signaling pathways in the adult rodent brain. Interestingly, TrkB and GSK3β signaling are similarly regulated by various pharmacologically different sedatives (but not with stimulant amphetamine) but, as expected based on huge amount of previous literature, these responses are not (always) co-associated with antidepressant-like behavioural and functional changes. Moreover, ketamine dose-dependently regulates TrkB and GSK3β signaling. Collectively these intriguing observations suggests that increased neuronal inhibition - rather than excitation - readily induce some of the intracellular signaling alterations intimately connected with rapid-acting antidepressant responses in the adult rodent brain. Obtained data urge to rethink and update some of the current antidepressant hypotheses and will guide subsequent experiments to find shared neurobiological mechanisms underlying rapid antidepressant actions.

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Poster

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: Brain Resource Company Operations Pty Ltd

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Title: Human amygdala engagement moderated by early life stress exposure is a biobehavioral target for predicting recovery on antidepressants

Authors: *A. N. GOLDSTEIN-PIEKARSKI[1,2], M. S. KORGAONKAR[4], E. GREEN[1,3], T. SUPPES[1,3], A. F. SCHATZBERG[1], T. HASTIE[5], C. B. NEMEROFF[6], L. M. WILLIAMS[1,2]; [1]Psychiatry and Behavioral Sci., Stanford Univ. Sch. of Med., Palo Alto, CA; [2]Sierra-Pacific Mental Illness Research, Education, and Clin. Ctr. (MIRECC), [3]Veterans Affairs Palo Alto Hlth. Care Syst., Palo Alto, CA; [4]Brain Dynamics Ctr., Univ. of Sydney Med. School-Westmead and The Westmead Inst. for Med. Res., Sydney, Australia; [5]Statistics Dept., Stanford Univ., Stanford, CA; [6]Psychiatry and Behavioral Sci., Univ. of Miami Miller Sch. of Med., Miami, FL

Abstract: Amygdala circuitry and early life stress (ELS) are both strongly and independently implicated in the neurobiology of depression. Importantly, animal models have revealed that the contribution of ELS to the development and maintenance of depression is likely a consequence of structural and physiological changes in amygdala circuitry in response to stress hormones. Despite these mechanistic foundations, amygdala engagement and ELS have not been investigated as biobehavioral targets for predicting therapeutic intervention outcomes in translational human studies of depression. To address this question 70 patients from the International Study to Predict Optimized Treatment in Depression (iSPOT-D) were scanned using fMRI when unmedicated, randomized to one of three antidepressants and re-assessed 8 weeks post-treatment. An established emotional face task probed amygdala engagement. ELS was measured with the Early Life Stress Questionnaire. Therapeutic outcome was assessed by a combined measure of symptoms (HAM-D and QIDS) and function (SOFAS). Hierarchical logistic regression and receiver operating characteristic analyses were used to test the predictive performance of models using amygdala engagement and ELS as targets for probing therapeutic outcome following an antidepressant intervention. The interaction between amygdala engagement by emotional stimuli and ELS predicted therapeutic functional remission on antidepressants with greater than 80% cross-validated accuracy. In depressed people exposed to high ELS, the likelihood of remission was highest when the amygdala was hyper-reactive to

socially rewarding stimuli, whereas for those with low ELS exposure, remission was predicted by a state of amygdala hypo-reactivity to both rewarding and threat-related stimuli. This full model predicted functional remission over and above the contribution of demographics, symptom severity, ELS and amygdala reactivity alone. These findings identify a human target for elucidating the mechanisms of antidepressant functional remission and offer a target for developing novel therapeutics. The results also offer a proof of concept for using neuroimaging as a target for guiding neuroscience-informed intervention decisions at the level of the individual person.

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Poster

735. Treatment and Drug Discovery: Depression

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

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Chicago Biomedical Consortium HTS-004

Title: Trip8 interaction with hcn regulates trafficking and antidepressant like behavior.

Authors: ***Y. HAN**, R. HEUERMANN, K. LYMAN, D. FISHER, Q.-A. ISMAIL, D. CHETKOVICH;
Northwestern Univ., Chicago, IL

Abstract: Major depressive disorder (MDD) affects millions of people worldwide. Most of the existing antidepressants target monoaminergic neurotransmitters, but many individuals don't respond to these therapies. As such, there is a need for new MDD treatments targeting novel mechanisms. Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels mediate I_h, an important current for controlling neuronal excitability. Brain HCN channels are tightly regulated by an auxiliary subunit, TRIP8b (tetratricopeptide repeat-containing Rab8b-interacting protein). Animals lacking either TRIP8b or HCN have increased antidepressant-like behavior, suggesting that inhibiting HCN channels could effectively treat depression. Unfortunately, directly targeting HCN channels throughout the body is not a viable therapeutic approach because these channels are critical in controlling heart rate. We reasoned that inhibiting brain-specific TRIP8b would be a safer way to target HCN channels and treat MDD. Here we employed an *in vivo* viral rescue approach in TRIP8b knockout mice to examine the function of HCN-TRIP8b interaction in mediating HCN channel trafficking and influencing antidepressant-like behavioral effects. We found that AAV-TRIP8b restored trafficking of HCN channels in TRIP8b KO mice. Furthermore, AAV-TRIP8b reversed the antidepressant-like behavioral phenotype of TRIP8b KO mice. We next generated a mutant TRIP8b construct in which binding to HCN subunits is impaired (AAV-TRIP8b (N13A)). Injection of AAV-TRIP8b (N13A) into the hippocampi of TRIP8b KO mice further decreased trafficking of HCN channels. As predicted by the hypothesis that HCN trafficking is important for behavior, AAV-TRIP8b (N13A) augmented the antidepressant-like behavioral phenotype of TRIP8b KO mice. These observations demonstrate that HCN channel trafficking bidirectionally regulates antidepressant-like behaviors and HCN-TRIP8b interaction might be a novel pharmacological target for MDD.

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Poster

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Title: Evaluation of scopolamine and muscarinic sub-type selective compounds in models of depression with greater translational value

Authors: *J. SHOBLOCK, W. A. ECKERT, III, W. CHEN, N. WELTY, G. CHEN; Neurosci., Janssen Res. & Development, L.L.C., San Diego, CA

Abstract: Recently, scopolamine has been shown to be a rapid-acting antidepressant in several clinical trials. This has initiated preclinical research to characterize the antidepressant-like effects of scopolamine, but so far such research has focused on immobility tests like forced swim. Immobility tests lack construct validity and respond acutely to antidepressants that require chronic clinical dosing. Therefore, we sought to characterize scopolamine in additional preclinical depression models, which have perhaps greater construct or predictive validity. We tested scopolamine in active avoidance, with either catecholamine depletion, serotonin depletion, or pre-exposure to inescapable shocks (the learned helpless model) used to induce a helpless-like state. Active avoidance test is typically insensitive to acute administration of traditional antidepressants, but responds to rapid-acting antidepressants like ketamine. Scopolamine significantly increased escapes in active avoidance test under all three conditions, indicating a rapid-acting antidepressant-like response. Post-test drug levels indicate effectiveness at 25 ng/ml in plasma and 67 ng/ml in brain in active avoidance. We then sought to use the learned helpless model to further characterize the mechanism of action of scopolamine's effect. Previous published data implicated the M2 subtype and we confirmed that an M2 antagonist was antidepressant-like in immobility test. This could be due to blockade of M2 autoreceptors, leading to increased acetylcholine and stimulation of nicotinic receptors, because the effect was blocked by the nicotinic antagonist mecamylamine and mimicked by a nicotinic a6b2/a4b2 agonist. However, mecamylamine did not block scoplamine's effect in learned helpless and the nicotinic agonist was without effect in learned helpless. On the other hand, an M5 positive allosteric modulator was able to attenuate scopolamine's effect in learned helpless. Thus, while M2 blockade may contribute to some antidepressant-like effect of scopolamine, it likely does not mediate the rapid-acting effects, whereas M5 may be involved. These data highlight the need for improved translational models when characterizing clinical drugs.

Disclosures: **J. Shoblock:** A. Employment/Salary (full or part-time): Janssen Research & Development, LLC. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Owns stock in Johnson & Johnson (JNJ). **W.A. Eckert:** A. Employment/Salary (full or part-time): Janssen Research & Development, LLC. **W. Chen:** A. Employment/Salary (full or part-time): Janssen Research & Development. **N. Welty:** A. Employment/Salary (full or part-time): Janssen Research & Development. **G. Chen:** A. Employment/Salary (full or part-time): Janssen Research & Development.

Poster

735. Treatment and Drug Discovery: Depression

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 735.17/GGG2

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Title: Effects of probiotic treatment (Bifidobacterium Infantis) in male and female rats exposed to chronic high levels of corticosterone

Authors: G. S. HAAS, S. M. MOONEY-LEBER, W. WANG, K. V. ZARIC, *S. BRUMMELTE;
Psychology, Wayne State Univ., Detroit, MI

Abstract: Previous research suggests that probiotics such as Bifidobacterium Infantis (B. infantis) may have beneficial health effects. The current study investigates whether B. infantis can alleviate depressive symptoms in a rodent model of depression. For this, 24 male and 24 female Sprague-Dawley rats received daily s.c. corticosterone (40mg/kg; to induce depressive-like behavior) or oil injections (control) coupled with voluntary consumption of a hazelnut cacao spread that was either pure (placebo) or mixed with one capsule of B. infantis for 21 days. Animals performed the Open Field Test and Forced Swim Test I and II on days 18, 20 and 21, respectively and several blood samples were collected to investigate basal as well as stress-induced corticosterone levels. Preliminary results revealed no significant difference in body weight or forced swim test behavior between the probiotic and placebo groups, but the expected decrease in body weight and increase in immobility in the Forced Swim Test in the corticosterone-treated animals. These results suggest that B. infantis may not be sufficient to improve depressive symptoms in our animal model. However, the effects on the brain and Hypothalamic-Pituitary-Adrenal axis are still under investigation and a trend for weight and behavior changes in females indicates that more research is needed to better understand potential sex difference concerning the health effects of probiotics.

Disclosures: G.S. Haas: None. **S.M. Mooney-Leber:** None. **W. Wang:** None. **K.V. Zaric:** None. **S. Brummelte:** None.

Poster

735. Treatment and Drug Discovery: Depression

Location: Halls B-H

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Program#/Poster#: 735.18/GGG3

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: ERA-NET HYPZITRP

Title: Hyperforin potentiates antidepressant-like activity of AZD 6765 in the tail suspension test in mice

Authors: B. POCHWAT, *B. SZEWCZYK, K. KOTARSKA, G. NOWAK;
Inst. of Pharmacol. PAS, Krakow, Poland

Abstract: Depression is one of the most debilitating medical problems concerning modern societies. Unfortunately, current pharmacological strategies in the treatment of depression are not sufficient, thus the new pharmacological solutions are desirable. Possible route to improve the pharmacotherapy of depression is the use of N-methyl-D-aspartate receptor (NMDAR) antagonists. These compounds have faster onset of action than classic antidepressants and have potential to be used in the acute episodes of depression or even in life threatening cases. However, the administration of NMDAR antagonists is limited because of their serious side effects. Thus, the strategies that help to decrease the doses of NMDAR antagonists or to extend duration of action of these compounds are needed. The aim of the present study was to potentiate the antidepressant-like activity of selected NMDAR antagonist by modulator of transient receptor potential cation channel, subfamily C, member 6, (TRPC6). As the pharmacological tools, we choose hyperforin which is well-known TRPC6 positive modulator and AZD 6765 which is a low trapping NMDAR antagonist. We have revealed that hyperforin in two doses (2,5 and 5 mg/kg) exerts its antidepressant-like activity 1 hour and 24 hours after administration in tail suspension test (TST) in mice. Similar effects have been observed for the AZD 6765 in the dose of 10 mg/kg. Furthermore, hyperforin enhanced the antidepressant-like effect of AZD 6765. In details, co-administration of non- effective dose of hyperforin (1 mg/kg, administered 1 hour before test) with non-effective dose of AZD 6765 (2,5 mg/kg, administered 1,5 h before test) showed antidepressant-like activity in TST. Additionally, co-administration of effective doses of hyperforin (2,5 or 5 mg/kg) with effective dose of AZD 6765 (10 mg/kg) increased duration of antidepressant effects for 72 hours. These results indicated that hyperforin, the positive modulator of TRPC6 may be a useful compound to potentiate the antidepressant activity of NMDAR antagonists.

Disclosures: B. Pochwat: None. **B. Szewczyk:** None. **K. Kotarska:** None. **G. Nowak:** None.

Poster

735. Treatment and Drug Discovery: Depression

Location: Halls B-H

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Program#/Poster#: 735.19/GGG4

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: MH/NS62391

MH089111

MH099851

Title: Disinhibition of somatostatin-positive interneurons produces antidepressant-like consequences in mice

Authors: *S. J. JEFFERSON[1], T. FUCHS[1], A. HOOPER[2], P. YEE[1], J. MAGUIRE[2], B. LUSCHER[1];
[1]The Pennsylvania State Univ., University Park, PA; [2]Tufts Univ. Sch. of Med., Boston, MA

Abstract: Major Depressive Disorder (MDD) is associated with deficits in the inhibitory neurotransmitter γ-aminobutyric acid (GABA) and antidepressant treatment can normalize these deficits. $GABA_A$ receptor-deficient mice exhibit a depressive-like phenotype that is normalized by conventional antidepressant treatment as well as subanesthetic ketamine injection. We hypothesized that enhancing GABAergic transmission in mice through disinhibition of somatostatin (SST)-positive interneurons, a subset of GABAergic interneurons that has been implicated in depressive disorders, would be sufficient to produce antidepressant-like behavioral consequences. To this end, we deleted the γ2 subunit gene of the $GABA_A$ receptor (*Gabrg2*) selectively in SST^+ interneurons (SSTCre:$\gamma2^{f/f}$ mice). To facilitate recordings from SST^+ interneurons we further crossed these mice with a Rosa26-YFP Cre-reporter (SSTCre:$\gamma2^{f/f}$ x Rosa26-YFP). A reduction in inhibitory input to SST^+ cells was confirmed by voltage clamp analysis, which showed a decrease in frequency and amplitude of spontaneous inhibitory postsynaptic currents (sIPSCs). SST^+ cells also showed increased excitability, as evidenced by an increase in input resistance and in the number of current-evoked action potentials. Consistent with potentiation of inhibitory synapses of target cells, we observed an increase in sIPSCs in pyramidal cells in L2/3 of the cortex and the hippocampal CA1 region. As predicted, the behavior of these mice mimicked the effect of anxiolytic and antidepressant drugs. Similar to mice treated with rapidly-acting antidepressants, SSTCre:$\gamma2^{f/f}$ mice showed reduced phosphorylation of the eukaryotic elongation factor eEF2 in extracts from the hippocampus and medial prefrontal cortex (mPFC). However, unlike mice treated with some rapidly-acting antidepressant drugs, the altered eEF2 phosphorylation observed in these mice was independent of altered mTOR signaling. Finally, SST mRNA and protein levels were unaltered in brain extracts from SSTCre:$\gamma2^{f/f}$ mice, indicating that potentiation of GABAergic synapses on principal cells alone is sufficient to produce an antidepressant-like behavioral and biochemical phenotype.

Disclosures: **S.J. Jefferson:** None. **T. Fuchs:** None. **A. Hooper:** None. **P. Yee:** None. **J. Maguire:** None. **B. Luscher:** None.

Poster

735. Treatment and Drug Discovery: Depression

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Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 735.20/GGG5

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Title: Specificity of the discriminative stimulus properties of the noncompetitive N-Methyl-D-aspartate (NMDA) receptor antagonist in C57BL/6 mice

Authors: *J. H. PORTER, S. E. CARLAN, S. E. YOUNG, T. J. BRAXTON, M. H. KHAN, H. NANGUNURI, H. NANGUNURI, R. PANDEY, C. W. KALINOWSKI, K. W. LOVELESS, D. SMITH, K. A. WEBSTER;
Psychology, Virginia Commonwealth Univ., Richmond, VA

Abstract: The noncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist ketamine has been shown to produce both rapid and long-lasting antidepressant effects in patients with major depressive disorder, including treatment-resistant patients; however, its abuse liability limits its outpatient use. Identification of the receptor mechanisms that mediate ketamine's therapeutic effects could help lead to development of novel drug treatments for depression. Using a two-lever drug discrimination procedure, we previously reported that the discriminative stimulus properties of ketamine are shared by other NMDA antagonists (phencyclidine, MK-801 and memantine), but not by the antidepressants imipramine and fluoxetine, indicating that ketamine's interoceptive effects appear to be independent of its antidepressant-like properties. In the present study selective receptor ligands and drugs from other therapeutic classes were tested to further explore the underlying mechanisms that mediate ketamine's discriminative stimulus properties. Male C57BL/6 mice were trained to discriminate 10 mg/kg of ketamine from vehicle in a two-lever drug discrimination task (10 min injection time, SC). Mice rapidly acquired the ketamine discrimination and a generalization curve (2.5 mg/kg - 20.0 mg/kg) was conducted and yielded an ED_{50} = 4.38 mg/kg (95% = CI 4.03 mg/kg-4.76 mg/kg). Substitution testing was conducted with raclopride (dopamine $D_{2/3}$ antagonist), quinpirole ($D_{2/3}$ agonist), d-amphetamine (dopamine reuptake inhibitor), quipazine (serotonin [5-HT] agonist), ritanserin (5-HT_2 antagonist), ketanserin (5-HT_{2A} antagonist), scopolamine (cholinergic muscarinic antagonist), pyrilamine (H_1 histamine antagonist), prazosin ($alpha_1$ adrenoceptor antagonist), yohimbine ($alpha_2$ adrenoceptor antagonist), methadone (opioid mu agonist), naloxone (opioid mu antagonist), dextrorphan (NMDA antagonist), and the benzodiazepine chlordiazepoxide ($GABA_A$ positive modulator). None of the selective ligands substituted for ketamine's discriminative stimulus, nor did chlordiazepoxide. The only compound that produced ketamine-appropriate responding was the NMDA antagonist dextrorphan. It produced 85.8% ketamine-lever responding at 30 mg/kg. These findings replicate our previous report that only NMDA antagonists mimic ketamine's discriminative stimulus, and also replicate findings in ketamine discrimination studies with rats.

Thus, ketamine's discriminative stimulus properties appear to be primarily mediated by antagonism of glutamatergic NMDA receptors.

Disclosures: **J.H. Porter:** None. **S.E. Carlan:** None. **S.E. Young:** None. **T.J. Braxton:** None. **M.H. Khan:** None. **H. Nangunuri:** None. **H. Nangunuri:** None. **R. Pandey:** None. **C.W. Kalinowski:** None. **K.W. Loveless:** None. **D. Smith:** None. **K.A. Webster:** None.

Poster

735. Treatment and Drug Discovery: Depression

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Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 735.21/GGG6

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: MoodNote LLC

Title: Estimation of response probabilities to 10 common antidepressants based on the geographic distribution of Single Nucleotide Polymorphisms (SNPs) associated with treatment responses

Authors: S. POSPOS, *A. BASKYS;
Riverside Psychiatric Med. Group, Riverside, CA

Abstract: Antidepressant selection on a "trial and error" basis often leads to poor treatment outcomes, significant adverse effects and poor treatment adherence. While genetic polymorphisms can influence antidepressant responses, their prevalence varies across geographic areas. Here we calculated response probabilities to 10 common antidepressants based on antidepressant response associated SNP distribution among CEU, MXL, ASW and CHB populations (population definitions from HapMap.org). SNPs associated with antidepressant responses were rs7997012 for 5-hydroxytryptamine (serotonin) receptor 2A or HTR2A (venlafaxine XR), rs2770296 for HTR2A (bupropion), rs352428 for FK506 binding protein 5 or FKBP5, rs1954787 for glutamate receptor ionotropic kainate 4 or GRIK4 (citalopram), rs61888800 for brain-derived neurotrophic factor or BDNF, rs25531 for solute carrier family 6 (neurotransmitter transporter, serotonin) member 4 or SLC6A4, rs242941 for corticotrophin-releasing hormone receptor 1 or CRHR1 (fluoxetine), rs10042486 and rs1364043 for 5-hydroxytryptamine (serotonin) receptor 1A or HTR1A, rs6314 for HTR2A, rs2472304, rs2470890, and rs4646425, rs762551, and rs4646427 for cytochrome P450, family 1, subfamily A, polypeptide 2 (CYP1A2), rs6265 for BDNF (paroxetine), rs10042486 and rs1364043 for HTR1A, rs4680 for catechol-O-methyltransferase or COMT (fluvoxamine), rs6265 for BDNF (mirtazapine), rs352428 for FKBP5 (escitalopram), rs10042486 and rs1364043 for HTR1A

(milnacipran) and rs61888800 for BDNF (desipramine) [Baskys 2015]. For each antidepressant response associated SNP, we calculated allele frequencies associated with the increased response.
CEU and MXL were most likely to respond to bupropion, ASW to mirtazapine and CHB to desipramine. The highest response probability for each antidepressant was: CEU, fluvoxamine (0.44), milnacipran (0.42); MXL, milnacipran (0.42); ASW, mirtazapine (1.00), bupropion and escitalopram (0.98), venlafaxine XR (0.95), paroxetine (0.60) and milnacipran (0.42); and CHB, desipramine (0.98), fluoxetine (0.88) and citalopram (0.72). The highest response probability for each population was fluvoxamine and milnacipran (CEU), milnacipran (MXL), mirtazapine, bupropion, escitalopram, venlafaxine XR, paroxetine and milnacipran (ASW) and desipramine, fluoxetine and citalopram (CHB).

Disclosures: **S. Pospos:** None. **A. Baskys:** 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; MoodNote LLC.

Poster

735. Treatment and Drug Discovery: Depression

Location: Halls B-H

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Program#/Poster#: 735.22/GGG7

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: Anna Licia Giovanetti Award from University of Pavia

Title: Bioenergetics of Fluoxetine: a functional proteomic study

Authors: *F. FERRARI, A. GORINI, R. F. VILLA;
Dept. of Biol. and Biotech., Univ. of Pavia, Pavia, Italy

Abstract: BACKGROUND - Neuroimaging studies on depressed patients showed brain energy metabolism abnormalities, followed by the normalization of cerebral bioenergetics after antidepressants (ADs) treatment [1]. However, in some experimental studies, ADs are inhibitors of mitochondrial function, while in others they have positive effects on mitochondrial energy metabolism. These conflicting results are likely due to macro-heterogeneity of brain areas and to ADs differential effects on pre-synaptic and on post-synaptic terminals, not considered before when evaluating ADs effects on brain mitochondria.
METHODS - The effects of 21-day treatment with the selective serotonin reuptake inhibitor Fluoxetine (10 mg/kg, i.p.) were evaluated on energy metabolism of rat frontal cerebral cortex.

Two populations of intra-synaptic mitochondria ("light" - LM; "heavy" - HM) were isolated according to Villa et al. [2] and the following enzyme activities have been assayed: citrate synthase (CS), succinate dehydrogenase (SDH), malate dehydrogenase (MDH) for Krebs' cycle; NADH-cytochrome c reductase (CCR), cytochrome oxidase (COX) for Electron Transport Chain; glutamate-oxaloacetate transaminase (GOT), glutamate-pyruvate transaminase (GPT) for glutamate and related amino acids metabolism.
RESULTS - In controls, SDH, COX, GOT and GPT activities were higher in LM *versus* HM, whose metabolic individuality is reflected by enzyme kinetics, as previously shown in physiological aging, experimental physiopathology and pharmacological treatments [3, 4]. Fluoxetine treatment (1) decreased MDH, SDH and GPT activities in LM; (2) enhanced COX and (3) decreased GPT activities in HM.
CONCLUSIONS - Fluoxetine modified the catalytic properties of energy-linked enzymes differentially respect to the types of intra-synaptic mitochondria, explaining at subcellular level the conflicting results about the effects of ADs on mitochondria reported in Literature. Fluoxetine exerted selective effects on the energy metabolism of pre-synaptic terminals, coherent with those previously obtained after Desipramine treatment. From a bioenergetic point of view, these results integrate pharmacodynamic features Fluoxetine. This study will proceed evaluating the effects of Dsipramine and Fluoxetine on other brain areas.
REFERENCES - [1] Moretti et al 2003. *Mol Psychiatry*, 8:773-85; [2] Villa et al 1989. *Cell Mol Neurobiol,* 9:247-62; [3] Villa et al 2012. *Neuroscience*, 227:55-66; [4] Villa et al. 2013. *Neurochem Int*, 63:765-81.
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Poster

735. Treatment and Drug Discovery: Depression

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Title: Examining the influence of aerobic exercise and bilateral movement on frontal alpha asymmetry

Authors: *R. A. HICKS, W. E. MCILROY;
Kinesiology, Univ. of Waterloo, Waterloo, ON, Canada

Abstract: Aerobic exercise has been commonly associated with decreased risk of developing major depression. The neurophysiological mechanisms by which this occurs are not fully understood and are key for fully utilizing aerobic exercise as a potential intervention for the disorder. One common neurophysiological marker associated with depression risk is the frontal alpha asymmetry (FAA). FAA is a ratio of resting brain activity between the left and right PFC measured via alpha frequency (8-13 Hz), which relate to approach and withdrawal motivational networks respectively. Previous research has shown that aerobic exercise, independent of load, positively influences resting state FAA to promote activity less associated with depression risk. The fact that changes in FAA are observed after low level exercise intensities raises the possibility that this influence on cortical activity is linked to movement rather than the cardiorespiratory demand. This work aimed to determine if effects of exercise on FAA observed was due to the physiological demand of exercise or rather the bilateral rhythmic movement that is common across exercise modes such as running or cycling. The specific purpose of this study was to investigate the influence of a moderate aerobic exercise bout and a matched "no load" bilateral movement task on FAA. Twelve young healthy subjects (5M, 7F; age 22.3 ±3) underwent three sessions in which they performed an experimental condition of either: 1) moderate aerobic exercise, 2) "no load" bilateral rhythmic movement or 3) sitting rest. FAA was measured pre intervention and 6-38 minutes post intervention. Results showed aerobic exercise significantly increased FAA at 22-38 minutes post exercise compared to pre exercise FAA levels. There was no significant effect of bilateral movement on FAA at any time period. These results suggest that in order for physical activity to cause a beneficial change in FAA, it must require some degree of physiological effort or demand to complete.

Disclosures: R.A. Hicks: None. **W.E. McIlroy:** None.

Poster

735. Treatment and Drug Discovery: Depression

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Program#/Poster#: 735.24/GGG9

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Title: Acute administration of a selective serotonergic reuptake inhibitor suppresses functional connectivity in the frontal-striatal circuit.

Authors: *H. T. HAMADA[1], K. HIKISHIMA[2], N. TAKATA[3], Y. SAKAI[4], K. TANAKA[3], K. DOTA[1];
[1]Neural Computation Unit, Okinawa Inst. of Sci. and Technol., Kunigami-Gun, Japan; [2]Okinawa

Inst. of Sci. and Technol., Okinawa, Japan; [3]keio Univ., Mita, Japan; [4]Kyoto Prefectural Univ. of Med., Kyoto, Japan

Abstract: Serotonin is a crucial neuromodulator which plays multiple roles including the regulation of mood. Human resting-state fMRI (rs-fMRI) studies revealed that serotonergic drugs such as selective serotonin reuptake inhibitors (SSRIs) decrease functional connectivity (FC) among brain regions regulating mood. While chronic administration of serotonergic drugs reduces the symptoms, such as loss of pleasure and depressive mood, in depression patients, their acute administration sometime worsens the symptoms. Here we hypothesize that acute administration of serotonergic drugs changes functional connectivity among mood-related brain regions under resting-state and test it using 11.7 tesla MRI scanner with a cryoprobe (Brucker Biospin) with healthy male C57BL/6J mice (voxel size: 150x150x300 (μm); TR: 3.0(s); imaging time per session: 10 (min)). Two groups were prepared (n=7 for each group). A group was administered with a SSRI, escitalopram (10mg/kg) resolved in saline, while another group was administered with only saline. For each group, we administered SSRI or saline one hour prior to imaging. Seed-based FC analysis was conducted with functional connectivity toolbox (CONN) using 20 seed regions such as the Right/Left amygdala, the Right/Left anterior cingulate cortex (ACC), the Right/Left orbitofrontal cortex, and the Right/Left caudate putamen (CPu) from the Australian Mouse Brain Mapping Consortium (AMBMC). In line with our hypothesis, FC of the left lateral orbital cortex (LO) with the right CPu and right dorsolateral orbital cortex (DLO) was significantly reduced ($p<0.05$, positive false discovery rate for multiple tests (p-FDR)). Reduction of FC between the right CPu and the right ventral orbital cortex (VO) was also found ($p<0.05$, p-FDR). Reduction of functional connectivity in the frontal-striatal circuit was observed. This concurs with the reduced FC of the circuit in mental disorders including the major depression. The frontal-striatal circuitry is known as a pivotal circuit for reward processing and motivation. Reduction of the FC in the circuit indicates that acute administration of serotonergic drugs suppresses reward processing and motivation, and this suppression might be a mechanism causing loss of pleasure under acute administration of serotonergic drugs.

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Poster

735. Treatment and Drug Discovery: Depression

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: NSFC 81302202

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NSFC 81441111

NSF-Shandong ZR2011HQ055

FODTS2015

Title: Reversal effects of the antidepressant desipramine on resistance of U251/TR cells to temozolomide

Authors: J. MA[1], Y.-R. YANG[1], J.-J. LIU[1], F.-F. LI[1], M.-H. CHEN[1], H. WANG[1], L. WANG[1], L.-L. SUN[1], F.-Z. WANG[2], D.-C. WANG[1], *H. ZHANG[3,1];
[1]Inst. of Pharmacology, Taishan Med. Univ., Taian, Shandong 271016, China; [2]Sch. of Life Science, Taishan Med. Univ., Taian, Shandong 271016, China; [3]Depts Behav Med. & Psych, Pharm, West Virginia Univ. Hlth. Sci. Ctr., Morgantown, WV

Abstract: Temozolomide (TMZ) is the most effective chemotherapeutic agent for treatment of glioma. However, the efficacy of glioma treatment is severely reduced by the resistance to TMZ. Since depressive disorders are common comorbidity in patients with advanced cancer, antidepressants have been considered as conventional adjuvant agents for treatment of cancer comorbid with depression. Here we examined the effect of desipramine (DMI) on resistance of U251/TR cells to temozolomide (TMZ) and the related mechanism. Incubation of U251/TR cells with DMI (20-80 μM) or TMZ (0.5-10 mM) alone for 24 h inhibited the cell growth with the IC50s 33.4 (± 2.17) μM and 2.5 (± 0.19) mM, respectively (r^2=0.983,0.982,$P < 0.05$). This was significantly potentiated by combined treatment with TMZ (1 mM) and DMI (30 μM), indicating synergistic cytotoxicity, as also demonstrated by significant nuclear fragmentation and condensation following combined treatment with both drugs. In addition, DMI and TMZ in combination produced apoptosis in U251/TR cells, which was attenuated by knockdown of CHOP using specific short interfering RNAs (siRNAs) that target CHOP. The results suggest that DMI reverses resistance of U251/TR cells to TMZ through activation of the CHOP-dependently apoptosis pathway. The current study provides a primary basis for treatment of advanced glioma cancer, particularly with comorbidity of depression, using combination of antidepressants such as DMI and anti-tumor drugs such as TMZ.

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Poster

735. Treatment and Drug Discovery: Depression

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: Research foundation of new York and NIH Grant 1R01DA035923

Title: The role of brain fatty acid binding protein on sucrose consumption and the forced swim test

Authors: *J. HAMILTON[1], B. CLAVIN[1], C. KOUMAS[1], S. HAJ-DAHMANE[1], D. DEUTSCH[2], M. KACZOCHA[3], P. K. THANOS[1];
[1]Res. Inst. on Addictions, SUNY Buffalo, Buffalo, NY; [2]Biochem., [3]Anesthesiol., Stony Brook Univ., Stony Brook, NY

Abstract: Recent research on the mechanisms of intracellular transport of the endocannabinoid anandamide by Fatty Acid Binding Proteins (FABPs) and subsequent development of SBFI26, (a pharmacological inhibitor of two brain-specific FABPs 5/7) has shown increases in extracellular anandamide. The goal of this study was to examine the role of FABPs on sucrose reward and depressive-like behavior in wild type and FABP5/7 deficient mice. Results showed that acute SBFI26 administration did not have any effect on sucrose preference or consumption in male mice. Similarly, SBFI26 treatment did not have any significant difference (compared to vehicle) in the forced swim test (FST). Male and female FABP 5/7 deficient mice, however, showed significant increases in sucrose consumption compared to their WT counterparts (21.0% and 19.5% increases, respectively). Assessment of the FST data revealed that FABP5/7 deficient mice showed overall lower immobility time. The fact that such differences were seen between the acute pharmacological approach and the genetic approach (gene deletion) of FABP inhibition needs to be further investigated. It may point to the actions of oleoylethanolamide (OEA) and/or palmitoylethanolamide (PEA) which activate the nuclear peroxisome proliferator-activated receptor α (PPARα); these related *N*-acylethanolamines (NAEs) have been previously reported to be elevated in FABP KO animals, but not with SBFI26 administration. These findings help characterize the behavioral profile for inhibiting FABPs on sucrose reward and depression. Further research will be needed to identity the individual contributions FABPs have on AEA, OEA, and PEA in reward-related functioning.

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Poster

736. Treatment of Depression: Ketamine

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NARSAD Young Investigator Grant from the Brain & Behavior Research Foundation, P&S Investigator

Barnard Summer Research Institute

Title: Hippocampal NR2B-containing NMDA receptors are critical for the antidepressant actions of ketamine

Authors: *C. T. LAGAMMA[1], J. C. MCGOWAN[1,2], S. C. LIM[3], D.-O. SEO[4], M. R. DREW[4], R. A. BRACHMAN5[5], C. A. DENNY[3,6];
[1]Barnard Col. of Columbia Univ., New York City, NY; [2]Doctoral Program in Neurobio. and Behavior, Columbia Univ., New York, NY; [3]Div. of Integrative Neurosci., New York State Psychiatric Inst. (NYSPI) / Res. Fndn. for Mental Hygiene, Inc. (RFMH), New York, NY; [4]Ctr. for Learning and Memory, Univ. of Texas at Austin, Austin, TX; [5]Gertrude H. Sergievsky Ctr., Columbia Univ. Med. Ctr., New York, NY; [6]Dept. of Psychiatry, Columbia Univ., New York, NY

Abstract: Background: Major depressive disorder is one of the most prevalent and pervasive mental illnesses that affect our population today. Current antidepressant treatment often takes weeks to have an effect, and many patients do not show substantial clinical improvement. Ketamine, an N-methyl-D-aspartic acid receptor (NMDAR) antagonist, has been shown to have rapid, long-lasting antidepressant effects in treatment-resistant patients. Here, we investigate whether ketamine's efficacy as an antidepressant is dependent on adult hippocampal neurogenesis in concert with the NR2B subunit of the NMDA receptor.
Methods: We utilized a transgenic approach to ablate the NR2B subunit of NMDAR's in 6-week-old adult-born hippocampal neurons in mice. We used two additional models to confirm ablation effects on 6-week-old adult born hippocampal neurons: x-irradiation and a GFAP-thymidine-kinase (TK) genetic model. Mice with or without NR2B were then administered either

saline or ketamine. We assessed antidepressant effects of ketamine in the forced swim test (FST), contextual fear conditioning (CFC), and novelty suppressed feeding (NSF) paradigms.
Results: Consistent with previous findings, we found that in mice containing NR2B, ketamine had antidepressant effects in FST, pro-cognitive effects in CFC, and anxiolytic effects in NSF. Deletion of NR2B from 6-week-old adult-born neurons occluded all these effects. Whole cell ablation in the genetic or x-irradiation models prevented some of ketamine's effects.
Conclusions: These data suggest that 6-week-old adult-born hippocampal neurons expressing NR2B are critical for modulating ketamine's rapid antidepressant response.

Disclosures: C.T. LaGamma: None. **J.C. McGowan:** None. **S.C. Lim:** None. **D. Seo:** None. **M.R. Drew:** None. **R.A. Brachman5:** None. **C.A. Denny:** None.

Poster

736. Treatment of Depression: Ketamine

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: NARSAD Young Investigator Award

Golden Rule Family Foundation

Title: Longitudinal effects of ketamine on dendritic architecture *In vivo* in the mouse medial frontal cortex

Authors: *A. C. KWAN, V. PHOUMTHIPPHAVONG, F. BARTHAS, S. HASSETT; Psychiatry, Yale Sch. of Med., New Haven, CT

Abstract: A single subanesthetic dose of ketamine, an NMDA receptor antagonist, leads to fast-acting antidepressant effects. In rodent models, systemic ketamine is associated with higher dendritic spine density in the prefrontal cortex, reflecting structural remodeling that may underlie the behavioral changes. However, turnover of dendritic spines is a dynamic process in vivo , and the longitudinal effects of ketamine on structural plasticity remain unclear. The purpose of the current study is to use subcellular resolution optical imaging to determine the time course of dendritic alterations in vivo following systemic ketamine administration in mice. We used two-photon microscopy to visualize repeatedly the same set of dendritic branches in the mouse medial frontal cortex (MFC) before and after a single injection of ketamine or saline. Compared to controls, ketamine-injected mice had higher dendritic spine density in MFC for up to 2 weeks. This prolonged increase in spine density was driven by an elevated spine formation rate, and not

by changes in the spine elimination rate. A fraction of the new spines following ketamine injection was persistent, which is indicative of functional synapses. In a few cases, we also observed retraction of distal apical tuft branches on the day immediately after ketamine administration. These results indicate that following systemic ketamine administration, certain dendritic inputs in MFC are removed immediately, while others are added gradually. These dynamic structural modifications are consistent with a model of ketamine action in which the net effect is a rebalancing of synaptic inputs received by frontal cortical neurons.

Disclosures: **A.C. Kwan:** None. **V. Phoumthipphavong:** None. **F. Barthas:** None. **S. Hassett:** None.

Poster

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: A*STAR - Joint Council Office (JCO) Development Platform (DP) Grant, Singapore

Title: In-vivo characterization of effects of psychoactive compounds, with novel therapeutic profile for MDD (ketamine and VU0409106) in a rodent model of anhedonia

Authors: *S. SAKTHIVEL[1], J. GARNELL[1], J. GRANDJEAN[1], A. THOMPSON[2], S. SERAMANI[1], C. LINDSLEY[2], C. JONES[2], K. BHAKOO[1];
[1]Singapore Bioimaging Consortium, A*Star, Singapore, Singapore; [2]Vanderbilt Ctr. for Neurosci. Drug Discovery, Vanderbilt Univ., Nashville, TN

Abstract: The current antidepressants for major depressive disorder (MDD) present a delayed therapeutic response, which can negatively impact compliance and/or have severe consequences for MDD patients suffering from suicidal ideations. We had previously characterised combination treatment strategies [SSRI's & SNRI's with 5-HT_{1A} & alpha2-adrenoceptors antagonists, resp.] to hasten antidepressant responses. Currently 'inhibition of NMDAR neurotransmission' represents an exciting & novel approach for rapid response, though the precise mechanism of action of such potential/novel psychoactive compounds are not yet fully characterised. Our drugs of interest were the NMDA antagonist, Ketamine (KET), an old drug but with new indications in MDD and VU0409106, a novel, potent & efficacious $mGlu_5$ negative allosteric modulator, which produces changes in signalling pathways comparable to KET, exemplified by its behavioural effects in our preclinical models of antidepressant-like activity. We have employed *in-vivo* MR Single Voxel Spectroscopy (SVS) to document the dynamic

neuro-metabolite changes (live flux profiles) over 2hrs, using ultra-high field 9.4T MRI, with & without these drug challenge. Male Wistar rats were used in this study; several cohorts went through a robust chronic mild unpredictable stress (CMUS) protocol. (I) CMUS led to reduced sucrose preference [CMUS: -41.3%, Naive: 0.05%, t-test: p=0.00022], reflecting a lack of reward-seeking behaviour (anhedonia). (II) CMUS led to marked change in metabolism: both glutamate (Glu) & glutamine (Gln) concentrations relative to total creatine were markedly decreased in CMUS compared to controls [Glu: p=0; Gln: p=2.7x10-9], consistent with the biogenic amine depletion hypothesis of MDD. Both inositol & total visible choline (glycerophosphocholine + phosphocholine) were increased in the CMUS group [Ins: p=4.4x10-13, GPC+PCh: p=0.0015], which may reflect inflammation & membrane metabolism changes respectively, consistent with our previous work in a mouse model of chronic social defeat. Less marked decreases in N-acetylaspartate [NAA: p= 0.0039] & Taurine [Tau: p=0.053] were also observed in CMUS compared to control. (III) Acute KET leads to Glu depletion over time in the control group specifically [Glu: p=0.00049]. (IV) Behaviourally KET & VU0409106 treatment increased the sucrose preference in CMUS animals compared to controls [CMUS+KET: 79%, CMUS+VU0409106: 74%, Naive: 48%, p<0.01 vs. CMS-Veh]. Our SVS results indicate a metabolic fingerprint similar to MDD; further documenting live flux profile with drug challenge offers novel insights, with high translational value to MDD clinical research.

Disclosures: **S. Sakthivel:** None. **J. Garnell:** None. **J. Grandjean:** None. **A. Thompson:** None. **S. Seramani:** None. **C. Lindsley:** None. **C. Jones:** None. **K. Bhakoo:** None.

Poster

736. Treatment of Depression: Ketamine

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: NIMH R21 MH100652

NIMH R01 MH053851

Title: A role for JAK2 signaling in ketamine-induced mTOR activation in the orbitofrontal cortex

Authors: *S. M. ADLER, M. PATTON, D. MORILAK, M. GIROTTI;
Pharmacol., Univ. of Texas Hlth. Hlth. Sci. Ctr. A, San Antonio, TX

Abstract: Because depression treatment is often ineffective and slow, ketamine's rapid antidepressant effects provide an exciting alternative. In the past, our lab has shown that acute injection of a low dose of ketamine reverses chronic-stressed induced deficits in cognitive flexibility mediated in the prefrontal cortex that model cognitive symptoms of depression. One such form of cognitive flexibility is reversal learning, mediated in the orbitofrontal cortex (OFC), which is compromised by chronic cold stress. We have further demonstrated that acute ketamine administration rapidly induces Janus Kinase 2 (JAK2) signaling in the OFC, and that this signaling is important for the therapeutic effect on reversal learning in chronically stressed rats. We are now investigating the mechanism by which JAK2 signaling in the OFC may mediate ketamine's therapeutic effects on reversal learning. Because ketamine is known to activate mammalian target of rapamycin (mTOR) in the pre-frontal cortex (PFC), we tested if this signaling molecule is also activated by ketamine in the OFC. We then tested a potential role of JAK2 in this activation.

In a cohort of female Sprague-Dawley rats, half underwent chronic intermittent cold stress treatment for 14 days. On day 15, they were given an acute injection of ketamine (10 mg/kg, i.p.) and sacrificed 90 min. OFC tissue was collected for western blot analysis. Ketamine induced a significant increase in the phosphorylation of ribosomal protein S6, a downstream marker of mTOR activation ($p<.05$). We also observed that the stressed female rats exhibited a trend toward a deficit in phosphorylated S6 levels, which was restored to non-stressed control levels by ketamine administration.

To determine if JAK2 participates in the activation of the mTOR pathway by ketamine, male Sprague Dawley rats were administered ketamine (10 mg/kg, i.p.) followed 10 min later by a JAK2 inhibitor, AG490 (10 mg/kg, i.p.). Two hr after ketamine administration, the animals were sacrificed for OFC tissue collection. We examined phosphorylation of S6 as well as Akt, a signaling protein that phosphorylates and activates mTOR. We observed a trend for AG490 to block the ketamine-induced increase of phosphorylated S6. In addition, JAK2 inhibition significantly reduced ketamine-induced Akt phosphorylation ($p<.05$). These results show that ketamine activates the mTOR pathway in the OFC, and JAK2 participates in the ketamine-induced activation of mTOR.

We plan on pursuing this further by administering AG490 to male and female Sprague Dawley rats that undergo chronic intermittent cold stress.

Disclosures: **S.M. Adler:** None. **M. Patton:** None. **D. Morilak:** 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; H. Lundbeck A/S. F. Consulting Fees (e.g., advisory boards); Lundbeck Research, USA. **M. Girotti:** None.

Poster

736. Treatment of Depression: Ketamine

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Title: Phenotypic profiling of ketamine at different concentrations for its antidepressant signature

Authors: *O. H.-U. SCHROEDER, K. JÜGELT, B. BADER;
NeuroProof GmbH, Rostock, Germany

Abstract: Primary neuronal cultures on micro-electrode arrays have a long tradition and are well characterized and validated. There is a plethora of literature data documenting their physiological relevance in research and drug discovery. Neuronal cell cultures derived from murine and human stem cells are delivering increasing insights in neuronal micro-circuitry and are powerful tool in phenotypic drug screening. Here we studied ketamine and its effects in a frontal cortex micro-circuitry. Primary cell cultures show brain region-specific activity pattern which can be clearly distinguished by pattern recognition methods. We describe and analyze these activity patterns with 204 spike train parameters computed by our in-house software NPWaveX. In case of ketamine we performed concentration response experiments with 8 concentrations added to these neuronal cell cultures each in an accumulative manner. For each concentration we recorded in a stable phase of 30 minutes of spike train activity and calculated for these phases all 204 spike train parameter. Afterwards we performed a similarity analysis for these data records with our compound profile database of 40 approved clinical drugs at their estimated therapeutic concentrations. We separated our data records of ketamine data into low concentrations up to 100 nM and second for all above. With these two sets we performed a similarity analysis against our database. Ketamine showed clearly different induced activity patterns below 100 nM and above 100 nM. At high concentrations we saw similarities to NMDA antagonists and anesthetics. In the low concentration range we saw similarities mostly to antidepressants, which is in agreement with the discussion of ketamine and its anti-depressant action at low concentrations. As a control we tested PCP, which didn't show similarities to anti-depressant compounds, suggesting different effects on neuronal function. With this show case we demonstrate the power of phenotypic screening with the micro-electrode array technology and a comprehensive multi-variate data analysis. It still remains open to understand the functional changes of activity pattern by anti-depressants in greater detail. This we will study functional models of depression in the future.

Disclosures: **O.H. Schroeder:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroProof GmbH. **K. Jügelt:** None. **B. Bader:** None.

Poster

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Columbia University Summer Undergraduate Research Fellowship (SURF)

Title: Prophylactic ketamine reduces fear expression but does not facilitate extinction

Authors: *J. C. MCGOWAN[1,2], S. C. LIM[3], M. TSITSIKLIS[2], Y. NERIA[4,5], R. A. BRACHMAN[3,4], C. A. DENNY[3,4];
[1]Barnard Col. of Columbia Univ., New York, NY; [2]Doctoral Program in Neurobio. and Behavior, Columbia Univ., New York, NY; [3]Div. of Integrative Neuroscience, New York State Psychiatric Inst. (NYSPI) / Res. Fndn. for Mental Hygiene, Inc. (RFMH), New York, NY; [4]Dept. of Psychiatry, Columbia Univ., New York, NY; [5]Dept. of Epidemiology, Columbia Univ., New York, NY

Abstract: Background: Ketamine, an *N-methyl-D-aspartate (NMDA)* glutamate antagonist, has been reported to be an efficacious antidepressant for depression and posttraumatic stress disorder, and most recently, to be a prophylactic against stress-induced depressive-like behavior. It remains unknown, however, when ketamine should be administered relative to a stressor or depressive episode in order to maximize its beneficial effects. Furthermore, it is unknown if ketamine can be prophylactic against subsequent episodes. Here, we systematically tested the utility of ketamine relative to a fear experience in order to determine the best interval for

ketamine to be administered in order to reduce fear or to prevent subsequent aversive episodes.
Methods: Using a 3-shock contextual fear conditioning (CFC) paradigm, we tested if ketamine could alter how 129SvEv mice respond to fear. Mice were administered a single dose of saline or ketamine (30 mg kg^{-1}) at varying time points before or after CFC, extinction, or reinstatement.
Results: Mice administered prophylactic ketamine 1 week before, but not 1 month before, CFC training exhibited reduced freezing behavior when compared with mice administered saline. In contrast, ketamine administration following CFC or during extinction did not alter subsequent fear expression. Interestingly, mice administered ketamine 1 h before CFC exhibited increased freezing behavior when compared with mice administered saline.
Conclusions: These data indicate that ketamine can diminish the fear response when given as a prophylactic, but not when given immediately before or after an aversive episode. Therefore, ketamine may be most useful if administered in a vaccine-like fashion in order to protect against fear-inducing stimuli.

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Poster

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: IRP-NIMH-NIH

Title: BDNF and VEGF but not GDNF are associated with an anti-suicidal response to ketamine

Authors: L. E. NEWMAN, E. M. RICHARDS, E. D. BALLARD, D. A. LUCKENBAUGH, M. PARK, P. YUAN, R. MACHADO-VIEIRA, *C. A. ZARATE, JR;
Div. Intramural Res. Program, NIMH, Bethesda, MD

Abstract: BACKGROUND: The U.S. suicide rate has not significantly changed over the past 50 years, due, in part, to limited knowledge of suicide neurobiology. An emerging body of literature suggests that the N-methyl-D-aspartate receptor antagonist ketamine, which produces a fast-acting antidepressant effect, may also elicit a rapid anti-suicidal response. Brain-derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF) and glial-derived neurotrophic factor (GDNF) are altered in major depressive episodes and are thought to contribute to antidepressant response. Further, research suggests that plasma BDNF and VEGF levels may be associated with suicidal behavior. Given these findings, the present study aimed to

assess whether BDNF, VEGF and GDNF differed by anti-suicidal response to ketamine. METHODS: Fifty-three patients with treatment resistant depression (major depressive disorder or bipolar disorder types I or II) and current suicidal ideation received intravenous ketamine hydrochloride (0.5 mg/ kg) over 40 minutes. Suicidal ideation was assessed using the Hamilton Depression Rating Scale (HAMD) suicide item and replicated using Montgomery-Asberg Depression Rating Scale (MADRS) suicide item and the first five items of the Scale for Suicide Ideation (SSI5). Clinical assessments and blood draws occurred at 60 minutes before, 230 minutes after and one day after ketamine infusion. ELISAs were used to determine BDNF and VEGF plasma levels. Magnetic Luminex multi-analyte kit was used to measure GDNF plasma levels. T-tests were used to compare responders and non-responders across measurements where responders reported no suicidal thoughts after infusion. RESULTS: On the HAMD, baseline BDNF (p=.015) and VEGF (p=.009) were lower in anti-suicidal responders to ketamine at 230 minutes post-ketamine infusion. Responders had greater increases in BDNF (p=.005) and decreases in VEGF (p=.005) at 230 minutes. One day after infusion, responders had an increase in BDNF (p=.025). GDNF was not significantly different in responders versus non-responders to ketamine. Effect sizes suggested results of analyses using the MADRS suicide item and SSI5 were generally comparable to those using the HAMD. CONCLUSIONS: This study demonstrates that plasma levels of BDNF and VEGF but not GDNF were different in anti-suicidal responders to ketamine. While analysis is necessary to determine the mechanisms by which these molecules are altered, our results suggest that BDNF and VEGF require further investigation in the search for biomarkers related to suicide risk.

Disclosures: **L.E. Newman:** A. Employment/Salary (full or part-time): National Institute of Mental Health. **E.M. Richards:** A. Employment/Salary (full or part-time): National Institute of Mental Health. **E.D. Ballard:** A. Employment/Salary (full or part-time): National Institute of Mental Health. **D.A. Luckenbaugh:** A. Employment/Salary (full or part-time): National Institute of Mental Health. **M. Park:** A. Employment/Salary (full or part-time): National Institute of Mental Health. **P. Yuan:** A. Employment/Salary (full or part-time): National Institute of Mental Health. **R. Machado-Vieira:** A. Employment/Salary (full or part-time): National Institute of Mental Health. **C.A. Zarate:** A. Employment/Salary (full or part-time): National Institute of Mental Health. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Holds use patent for ketamine..

Poster

736. Treatment of Depression: Ketamine

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: NIGMS Grant GM64783

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Title: Desipramine interacts potently with MK-801, but not with ketamine in behavior

Authors: *J. A. TEMPLE, T. T. TOWNER, A. ROCHA, K. A. TRUJILLO;
California State Univ. San Marcos, San Marcos, CA

Abstract: Many patients suffering from major depressive disorder (MDD) are resistant to traditional antidepressant medications such as selective serotonin reuptake inhibitors or tricyclic antidepressants. The primary pharmacological action of traditional antidepressants is thought to be acting through blockade of monoamine reuptake, however, recent evidence suggests that other neurotransmitters may be involved. Glutamate is the most abundant neurotransmitter in the brain, and research supports the idea that N-methyl-D-aspartate (NMDA) glutamate receptors might play a role in the therapeutic effect of antidepressants. Ketamine, an NMDA receptor antagonist,has promising antidepressant effects in humans, including individuals resistant to currently available medications, and this has been replicated in animal models. Ketamine produces significant side effects and is abused by some individuals, so it is unlikely to replace traditional antidepressants. It is therefore important to better understand ketamine's antidepressant effects and develop alternatives. Past work in our laboratory and others has demonstrated that the combination of an NMDA receptor antagonist with a traditional antidepressant produces a profound increase in the locomotor stimulant effect of the NMDA antagonist. This action points toward potent interactions between traditional antidepressants and NMDA receptors. In the current study we examined the locomotor effects of ketamine or MK-801 in combination with desipramine (DMI), a tricyclic antidepressant, in Sprague Dawley rats. We hypothesized that a combination of an NMDA receptor antagonist and DMI would produce a stimulation of activity greater than either drug alone. MK-801 (0.1 mg/kg) alone produced a mild stimulant effect, and DMI (5.0 mg/kg) alone produced a slight locomotor depression when compared to the saline control group. As hypothesized, the stimulant effects of MK-801 were enhanced when combined
with DMI, supporting the idea that DMI interacts with glutamatergic systems. Ketamine (10 or 30 mg/kg) alone produced a short-lived stimulation of locomotor activity. However, in contrast to MK-801, DMI inhibited the locomotor stimulant effect of ketamine. The results suggest that ketamine differs from MK-801 and other NMDA receptor antagonists in its interaction with DMI. We are investigating potential explanations for the unique actions of ketamine.

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Poster

736. Treatment of Depression: Ketamine

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: National Science Council, Taiwan

Kun-Po Soo Medical Foundation, Taiwan

Title: Frontal EEG predictors for treatment outcome of subanesthetic - dose ketamine infusion in medication resistant depression: using wireless EEG

Authors: *T.-P. T. SU[1], Z.-H. CAO[2], M.-H. CHEN[3], C.-T. LI[3], C.-T. LIN[2];
[1]Veterans Gen. Hosp, Taipei, Taiwan; [2]Dept. of Electrical and Computer Engin. & BRC, Inst. of Electrical Control Engineering, Natl. Chia-Tong Univ., Hsin-Chu, Taiwan; [3]Psychiatry, Taipei Veterans Gen. Hosp, Taipei, Taiwan

Abstract: Objective: Intravenous infusion of low-dose ketamine, a glutamate N-methyl-D-aspartate (NMDA) receptor antagonist, was found to have a rapid and robust antidepressant effect in treatment resistance depression (TRD), which was paralleling with increased glucose metabolism in the frontal area by PET-FDG. The aim of the study is to investigate the role of frontal EEG as predictor for clinical response to Ketamine in treatment resistance depression (TRD) via a wireless EEG device. **Methods: p**atients received a randomized, controlled trial of a single dose of ketamine infusion, comparing the effect of active dose (0.2 ~ 0.5 kg/mg)(N=36, F/M=30/6, age: 47.0 ± 12.8 years) and placebo (normal saline)(N=18, F/M = 13/5, age ± SD: 51.0 ± 7.6 years). All patients received mood evaluation(HAMD_17) before and after infusion. Responder was identified (>= 50% reduction of baseline depression symptoms at 240 minutes post-infusion). Five - minute electroencephalography (EEG) was recorded via a wireless EEG device with 4 prefrontal dry-contact sensors in baseline (0 min) and post-treatment (240 min), respectively. EEG power and hemispheric asymmetry were calculated in the delta, theta, low alpha and high alpha bands. **Results:** In active dose group, we found 16 responders and 20 non-responders with HAMD_17 score reduction of 51% and 17%, respectively vs. 20% in the placebo. During pre-treatment, the EEG power analysis indicated that ketamine responders had lower relative theta and low alpha power relative to non-responders ($p < .05$). Using machine-learning technology, we classified responders and non-responders with 80.0 ± 9.8% accuracy, 81.5 ± 8.4 % sensitivity and 92.4 ± 7.6 % specificity based on EEG power features. Further, in the responders, we found that ketamine increased the low relative alpha power ($p<0.01$) and decreased its hemispheric (Fp2-Fp1) asymmetry over bilateral frontals ($p < .05$), which was observed in neither non-responders nor placebo controls. **Conclusion:** The results of this study provided the evidence of immediate changes of frontal activity may account for rapid clinical

response of ketamine and the pre-treatment frontal brain activity measured by simple EEG device might be a biomarker for better outcome prediction.

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Poster

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Title: Ketamine and S(+)-ketamine modulate the extracellular levels of monoamine- and amino acid neurotransmitters monitored by microdialysis in the prefrontal cortex and ventral hippocampus of awake rats

Authors: ***J. KEHR**[1,2], F. ICHINOSE[1], F.-H. WANG[1], S. SCHMIDT[1], S. YOSHITAKE[1], T. YOSHITAKE[2];
[1]Pronexus Analytical AB, Stockholm, Sweden; [2]Dept. of Physiol. and Pharmacol., Karolinska Institutet, Stockholm, Sweden

Abstract: Ketamine (Ketalar®) is currently evaluated in clinical trials for its efficacy in treatment of major depression. The objective of the study was to evaluate, by use of microdialysis, the effects of acute administration of racemic ketamine and its isomer S(+)-Ketamine on extracellular levels of neurotransmitters Glu, GABA, DA, NE, 5-HT and their acidic metabolites DOPAC, HVA and 5-HIAA in the medial prefrontal cortex (mPFC) and ventral hippocampus (vHPC) of awake rats and mice. Amino acids, monoamines, their metabolites were measured by liquid chromatography with tandem mass spectrometry (UHPLC-MS/MS). Both S(+)-ketamine (30 mg/kg) and ketamine increased the Glu levels to about 160% of controls at 40 min and 90 min, respectively. Similarly, the DA and NE levels increased to 178% and 145%, respectively, whereas the 5-HT and GABA levels were not affected. Concentrations of ketamine and S(+)-ketamine in the brain microdialysates were correlated to the levels of neurotransmitters allowing to estimate the PK/PD relationship.

Disclosures: **J. Kehr:** A. Employment/Salary (full or part-time): Employed by Pronexus. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; Principal investigator. **F. Ichinose:** A. Employment/Salary (full or part-time): Employee. **F. Wang:** A. Employment/Salary (full or

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Poster

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University of Dayton Council SEED Grant

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University of Dayton Graduate Program

Title: Repeated ketamine treatment induces sex-specific behavioral and neurochemical effects in stress-naive C57BL/6J mice

Authors: *C. THELEN[1], J. SENS[1], J. MAUCH[1], R. PANDIT[2], P. PITYCHOUTIS[1];
[1]Biol., Univ. of Dayton Dept. of Biol., Dayton, OH; [2]The Ohio State Univ., Columbus, OH

Abstract: One of the most striking discoveries in the treatment of major depression was the finding that infusion of a single sub-anesthetic dose of the N-methyl-D-aspartate (NMDA) receptor antagonist ketamine induces rapid and sustained antidepressant-like effects in treatment-resistant depressed patients and in animal models of depression. However, ketamine's antidepressant-like actions are transient and can only be sustained by repeated drug treatment. Despite the fact that women experience major depression at roughly twice the rate of men, research regarding the neurobiological antidepressant-relevant effects of ketamine has focused almost exclusively on the male sex. Notably, knowledge regarding the sex-differentiated effects, the frequency and the dose on which repeated ketamine administration stops being beneficial and becomes harmful, is limited. In the current study, we investigated the behavioral, neurochemical and synaptic molecular effects of repeated ketamine treatment (10 mg/kg; 21 days) in C57BL/6J mice of both sexes. We report that ketamine induced beneficial antidepressant-like effects in male mice, but induced both anxiogenic (i.e. decreased time spent in the center of the open field arena) and depressogenic effects (i.e. enhanced immobility duration in the forced swim test; FST) in their female counterparts. Moreover, repeated ketamine treatment induced sustained sex-differentiated neurochemical and molecular effects, as it enhanced hippocampal synapsin protein levels and serotonin turnover in males, but attenuated glutamate and aspartate levels in female

mice. Taken together, our findings indicate that repeated ketamine treatment induces opposite behavioral effects in male and female mice, and thus, present data have far-reaching implications for the sex-oriented use of this drug in both preclinical and clinical research settings.

Disclosures: **C. Thelen:** None. **J. Sens:** None. **J. Mauch:** None. **R. Pandit:** None. **P. Pitychoutis:** A. Employment/Salary (full or part-time): full-time employment.

Poster

736. Treatment of Depression: Ketamine

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 736.12/GGG23

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Title: Dissociating the antidepressant-like and abuse-related effects of ketamine by selective activation of the ventral hippocampus in rats.

Authors: *G. T. COLLINS[1,3], F. R. CARRENO[1,2], D. J. LODGE[1,2], A. FRAZER[1,2,3]; [1]Dept of Pharmacol., [2]Ctr. for Biomed. Neurosci., UT Hlth. Sci. Ctr. at San Antonio, San Antonio, TX; [3]South Texas Veterans Hlth. Care Syst., San Antonio, TX

Abstract: Despite very promising rapid and sustained antidepressant effects, the use of ketamine to treat refractory depression is limited by adverse effects, including those related to its abuse. Previously, we demonstrated that activation of a circuit from the ventral hippocampus (vHipp) to the medial prefrontal cortex is both necessary and sufficient for ketamine's sustained antidepressant-like effects in rats. In order to test the hypothesis that augmentation of hippocampal activity is capable of producing a sustained antidepressant-like response without also producing abuse-related effects, we evaluated effects of L-655,708, a negative allosteric modulator of alpha-5 GABAA receptors, as these receptors are selectively expressed in the hippocampus. Similar to ketamine, systemic administration of L-655,708 produced an antidepressant-like effect in the forced swim test that was apparent one week following a single administration. Transient bilateral inactivation of the vHipp by lidocaine at the time of L-655,708 administration prevented this effect, suggesting that hippocampal activity at the time of drug administration is necessary for its sustained antidepressant-like effect. Unlike ketamine, which disrupted the inhibition of acoustic startle response by a weak prepulse stimulus intensity, L-655,708 did not affect prepulse inhibition suggesting that it does not impair sensory-motor gating. In addition, when evaluated in rats trained to self-administer ketamine, L-655,708 failed to maintain responding at levels that were any different than vehicle suggesting that it would have no (or low) abuse liability. Taken together, these findings suggest that selective activation of the vHipp by a negative allosteric modulator of alpha-5 GABAA receptors is capable of

producing a sustained antidepressant-like effect in the absence of any psychotomimetic or abuse-related effects. By identifying pharmacological interventions that recapitulate the therapeutic effects of ketamine without its psychotomimetic and abuse-related effects, it should be possible to provide novel, safe, and effective approaches for treating patients suffering from refractory depression.

Disclosures: **G.T. Collins:** None. **F.R. Carreno:** None. **D.J. Lodge:** None. **A. Frazer:** None.

Poster

736. Treatment of Depression: Ketamine

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 736.13/GGG24

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: Department fund from Loma Linda University School of Pharmacy

Title: Ketamine inhibits pyramidal neurons in the prefrontal cortex in chloral-hydrate anesthetized rats

Authors: ***G. SHEN**, W.-X. SHI;
Dept. of Pharmaceut. and Administrative Sicences, Sch. of Pharm., Loma Linda Univ., Loma Linda, CA

Abstract: At subanesthetic doses, ketamine has been shown to be a fast acting antidepressant. However, ketamine is also psychotomimetic at the same doses. Evidences suggest that the prefrontal cortex (PFC) plays a major role in both the antidepressant and psychotomimetic effects of ketamine. To further understand the mechanism of action of ketamine, we investigated the effects of ketamine on PFC pyramidal neurons using in vivo single-cell recording in chloral hydrate-anesthetized male rats. PFC local field potentials (LFP) recorded from the same electrodes were also studied. We found that under baseline conditions, PFC LFP showed a pronounced slow oscillation (0.3-1.5 Hz) between the up and down states. The recorded pyramidal neurons fired only during PFC up states. Ketamine at doses starts from 1.25 mg/kg (i.v.) significantly reduced the firing activity of pyramidal neurons and decreased the duration of up states. Both effects were mimicked by the selective NMDA receptor channel blocker MK801 (0.125-1mg/kg), but not by Ro 25-6981(2.5-10mg/kg), an antagonist selective for NMDA receptors containing the NR2B subunit. The dopamine (DA) receptor antagonist fluphenazine (1mg/kg), previously shown to block the inhibition of PFC neurons induced by phencyclidine (an analogue of ketamine) (Gratton, Hoffer and Freedman. 1987), failed to prevent or reverse the effect of ketamine. These results suggest that ketamine inhibits pyramidal neurons by blocking

NMDA receptor, and the effect does not involve DA receptor activation. The finding that MK801 but not Ro 25-6981 mimicked the effect of ketamine further suggests that non-NR2B containing NMDA receptors play a major role in the inhibitory effect of ketamine on PFC pyramidal neurons.

Disclosures: G. Shen: None. **W. Shi:** None.

Poster

736. Treatment of Depression: Ketamine

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: HMRF 03143096

RGC/ECS 27103715

NSFC 31571031

Title: Effects of ketamine on dendritic spine plasticity in animal depression model

Authors: *C. S. LAI, L. H. L. NG, Y. HUANG, R. C. C. CHANG;
Sch. of Biomed. Sci., The Univ. of Hong Kong, Hong Kong, Hong Kong

Abstract: Depression is a mental disorder that is affecting 350 million people worldwide. Although the number of individuals with depression is constantly growing, the understanding of the pathogenesis of depression is still insufficient and effective treatments are lacking. Functional and structural deficits in the prefrontal cortex have been shown in major depressive disorder patients and chronic restraint stress (CRS) depression animal model, for example, retraction of dendrites, loss of dendritic spines, reduced expression of synaptic proteins, and altered synaptic transmission. Ketamine, a N-methyl-D-aspartate receptor antagonist, has gained interest as an antidepressant in recent years based on its rapid antidepressant effects in clinical studies. It has been proposed that ketamine enhances excitatory drive through disinhibition of excitatory pyramidal neurons by preferential suppression of parvalbumin interneurons and promotes synaptogenesis. However, the effects of ketamine on dendritic spine plasticity in animal depression model remain unclear. Here we used high resolution transcranial two-photon microscopy to investigate the effects of CRS and ketamine on the dendritic spine plasticity of layer V pyramidal neurons in the frontal association cortex. We found that CRS significantly increased the rate of spine elimination and reduced the rate of spine formation before the onset of depression-like behavioural symptoms. In addition, we found that ketamine reversed the effects

of CRS on dendritic spine plasticity. We will further investigate the potential involvement of parvalbumin interneurons in the antidepressant effects of ketamine on dendritic spine plasticity in the CRS depression model.

Disclosures: **C.S. Lai:** None. **L.H.L. Ng:** None. **Y. Huang:** None. **R.C.C. Chang:** None.

Poster

736. Treatment of Depression: Ketamine

Location: Halls B-H

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Program#/Poster#: 736.15/GGG26

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: University of Michigan Psychopharmacology Research Fund 361024

Title: Deciphering racemic ketamine, its two enantiomers mechanisms of analgesic, anesthetic and antidepressant actions

Authors: *E. F. DOMINO, M. HIRASAWA-FUJITA;
Univ. of Michigan, Ann Arbor, MI

Abstract: Racemic ketamine is well known as an N-methyl-D-aspartate (NMDA) receptor antagonist for its many useful clinical effects as well as a schizomimetic drug of abuse. Depending on its concentration or dose racemic ketamine also interacts with non-NMDA glutamate receptors, hyperpolarization-activated cyclic nucleotide channels, nicotinic, muscarinic, cholinergic and monoaminergic neuromodulation including serotonin and dopamine, kappa, delta and mu opioid receptors, nitric oxide/cGMP system, neurosteroids, and L-type Ca^{2+} channels. The concentrations of racemic ketamine for clinical and basic research studies both in vivo and in vitro very widely from 50 to 10,000 ng/mL (0.21 - 42.1 nmol/ml). Human anesthetic blood concentrations are greater than 2,000 with peak levels as high as 10,000 ng/ml. Patients return to consciousness ~1,000 ng/ml. Racemic ketamine with plasma concentrations of ~400 ng/ml via i.v. and as low as 40 ng/ml via oral administration has analgesic and antidepressant effects. Since 1963 S(+)-ketamine was found to be the more potent and effective enantiomer than R(-)-ketamine. However, in 2014, Zhang et al. (Pharmacology, Biochemistry and Behavior 116 (2014) 137-141) reported R(-)-ketamine in equal doses to be a more potent antidepressant in mice than S(+)-ketamine with no psychomotor effects.For low dose racemic ketamine, its NMDA antagonism and serotonin reuptake inhibition appear to be its major mechanisms of action. Compared to S(+)-ketamine, the less potent enantiomer R(-)-ketamine requires many additional studies for its analgesic and antidepressant effects.

Disclosures: **E.F. Domino:** None. **M. Hirasawa-Fujita:** None.

Poster

736. Treatment of Depression: Ketamine

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: NIH Grant MK099085

Bryan Robinson Endowment, Tallahassee Memorial Hospital

Title: Sex differences in the antidepressant-like effect of low dose ketamine in C57BL/6 mice

Authors: ***A. M. DOSSAT**, K. N. WRIGHT, C. E. STRONG, M. KABBAJ;
Biomed. Sci., Florida State Univ., Tallahassee, FL

Abstract: Females are twice as likely to suffer from depression as compared to males, with many experiencing mood disorder symptoms during periods of ovarian hormone fluctuations. In addition to disparity in depression prevalence among the sexes, there are also established sex differences in antidepressant response. The NMDA receptor antagonist, Ketamine (KET), has shown promise as a rapid-acting antidepressant, exerting effects within hours of treatment. There is a growing wealth of data regarding the neural mechanisms that mediate the rapid antidepressant effect of KET (e.g., BDNF and mTOR), yet sex differences in the molecular mechanisms of KET remain to be resolved. Our group previously reported that female rats are more sensitive to the antidepressant-like effect of KET, an effect mediated by the ovarian hormones estrogen (E2) and progesterone (P4). The present study aimed to: 1) determine if C57BL/6 mice display sex differences in KET sensitivity, 2) determine the role of endogenous ovarian hormones and their respective receptor subtypes to promote KET sensitivity, 3) and examine whether the same molecular mechanisms mediating ketamine antidepressant effects described previously in male rodents also apply to females. KET (0, 1.5, or 3 mg/kg, i.p.) was delivered 30 min prior to a forced swim test. Consistent with prior reports, male mice had reduced time immobile following 3 mg/kg KET. Females had reduced immobility after treatment with both 1.5 and 3 mg/kg KET; this increased sensitivity to KET was observed exclusively in proestrus (Pro, ovarian hormone peak) and not in diestrus females (D1, ovarian hormone nadir). We then treated D1 females with an agonist for ERα (PPT), ERβ (DPN), or P4 24 h prior to treatment with KET (1.5 mg/kg). Only PPT- and DPN-treated mice exhibited less time immobile when treated with KET, suggesting that E2 increases KET sensitivity via action at both ERα and ERβ. Since many of the rapid intracellular signaling cascades initiated by KET within the

hippocampus (HPC) (e.g., PI3K, mTOR) are also influenced by E2, we used Western blot to investigate their shared targets. Our analysis revealed an ovarian hormone-mediated sex difference in levels of KET-induced phosphorylation (p-) of Akt, p-CaMKIIα, and p-mTOR at the lowest KET dose but not at the highest dose. Together these data suggest that E2 increased behavioral sensitivity to KET at the lowest dose by enhancing KET effects on PI3K, CaMKIIα, and ultimately mTOR within the HPC to enhance translation of synaptic proteins. We are now conducting functional studies to firmly implicate some of these cascades and their targets in the E2-mediated enhancement in ketamine sensitivity.

Disclosures: **A.M. Dossat:** None. **K.N. Wright:** None. **C.E. Strong:** None. **M. Kabbaj:** None.

Poster

737. Neural Mechanisms of Value-Based Decision-Making

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 737.01/HHH2

Topic: H.01. Animal Cognition and Behavior

Title: Both D1 and D2 receptor-expressing striatal neurons encode positive as well as negative reward prediction error.

Authors: ***J. SHIN**, M. JUNG;
KAIST, Taejon-City, Korea, Republic of

Abstract: Basal ganglia are considered as a key structure for reward-based learning and voluntary motor control. Traditionally, D1 and D2 receptor-expressing striatal neurons have been considered to be segregated as direct and indirect pathways and play opposing roles in motor control and reward-based learning. However, recent studies have shown that the direct and indirect pathways are not clearly segregated and that the two pathways are activated concomitantly in association with movement initiation, suggesting complementary roles of the direct and indirect circuits in the functioning of the basal ganglia. In this study, under the hypothesis that the direct and indirect pathways are involved in learning from both positive and negative outcomes, we examined neural activity related to positive and negative reward prediction errors in each pathway. For this, we recorded responses of optogenetically-tagged D1 and D2 type medium spiny neurons (MSNs) in the dorsomedial striatum of D1 and D2 Cre mice, respectively, performing a probabilistic Pavlovian conditioning task. Some of D1 (10 out of 40, 25%) as well as D2 type (11 out of 39, 28%) MSNs showed significant linear response to positive reward prediction error in rewarded trials. In unrewarded trials, a larger proportion of D2 type MSNs (15 out of 39, 38%) responded significantly to negative reward prediction error compared to D1 type MSNs (7 out of 40, 17%; χ^2-test, p=0.0377). These results, although

preliminary, suggest that both D1 and D2 type MSNs encode positive as well as negative reward prediction errors, and that D2 type MSNs may play a more dominant role in signaling negative prediction error.

Disclosures: J. Shin: None. **M. Jung:** None.

Poster

737. Neural Mechanisms of Value-Based Decision-Making

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 737.02/HHH3

Topic: H.01. Animal Cognition and Behavior

Support: IBS-R002-G1

Title: Effect of dopamine depletion on reward prediction error signals in the striatum

Authors: *S. HUH[1,2], J. LEE[3], N. HUH[1,2], M. JUNG[1,2];
[1]Inst. for Basic Sci., Daejeon, Korea, Republic of; [2]Dept. of Biol. Sci., Korea Advanced Inst. of Sci. and Technol., Daejeon, Korea, Republic of; [3]Dept. of Neurobio., Howard Hughes Med. Institute,Harvard Med. Sch., Boston, MA

Abstract: Phasic discharges of midbrain dopamine neurons are correlated with reward prediction error (RPE). Traditionally, dopamine has been thought to broadcast RPE signals to different areas of the brain. However, previous studies have shown that signals necessary to computer RPE, namely chosen value and trial outcome signals, converge in dopaminoceptive regions of the brain, such as striatum and orbitofrontal cortex, suggesting local computation of RPE. To address this issue, we examined RPE-related neural activity in the dorsal striatum of rats performing a probabilistic Pavlovian conditioning task after depleting dopaminergic fibers with 6-hydroxy dopamine, which was confirmed by amphetamine-induced rotational bias. We found that the fraction RPE-coding striatal neurons in dopamine-depleted animals was no smaller than that in sham-lesioned animals. Our results support the view that RPE is computed locally in the striatum rather than being driven by dopaminergic inputs.

Disclosures: S. Huh: A. Employment/Salary (full or part-time): full. 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; IBS-R002-G1. **J. Lee:** None. **N. Huh:** None. **M. Jung:** None.

Poster

737. Neural Mechanisms of Value-Based Decision-Making

Location: Halls B-H

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Program#/Poster#: 737.03/HHH4

Topic: H.01. Animal Cognition and Behavior

Support: Research Center Program of the Institute for Basic Science 329 (IBS-R002-G1)

Title: Comparison of striatal and prefrontal neuronal activity during temporal discrimination

Authors: *J.-E. KIM[1], E. HER[2], M. JUNG[1];
[1]Korea Advanced Inst. of Sci. and Technol., Daejeon, Korea, Republic of; [2]Ajou Univ. Sch. of Med., Suwon, Korea, Republic of

Abstract: Even though the frontal cortex-basal ganglia circuit is known to play an important role in interval timing, it remains unclear how different components of this circuit contribute to interval timing. We have shown previously that some neurons in the rat medial prefrontal cortex (mPFC) convey temporal information in the form of monotonically changing (ramping) activity in a temporal bisection task, and that mPFC neuronal ensemble activity is tightly correlated with time interval discrimination behavior of the rat (Kim et al. 2013). In the present study, we examined neuronal activity in the dorsomedial and dorsolateral striatum in rats performing the same temporal bisection task and compared with the previously recorded mPFC neuronal activity. Few striatal neurons showed prolonged ramping activity found in the mPFC, and the majority were active only briefly during specific epochs of a sample interval. Also, compared to mPFC neurons, striatal neurons showed weaker tendency for logarithmic encoding of time, and their activity was less closely correlated with the animal's judgement of time. Our results show that the mPFC and striatum convey temporal information based on distinct underlying neural processes, and suggest that the mPFC plays a more important role than the striatum in controlling interval timing behavior.

Disclosures: J. Kim: None. **E. Her:** None. **M. Jung:** None.

Poster

737. Neural Mechanisms of Value-Based Decision-Making

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Program#/Poster#: 737.04/HHH5

Topic: H.01. Animal Cognition and Behavior

Support: Institute for Basic Science (IBS-R002-G1)

Title: Value-related activity and stimulation effects of parvalbumin-expressing neurons in the orbitofrontal cortex

Authors: ***Y. JANG**[1], S. JO[2], D. KIM[3], M. JUNG[1,2,3];
[1]Biol. Sci., Korea Advanced Inst. of Sci. and Technol., Daejon, Korea, Republic of; [2]Ctr. for Synaptic Brain Dysfunctions, Inst. for Basic science, Daejeon, Korea, Republic of; [3]Grad. Sch. of Med. Sci. and Engineering, Korea Advanced Inst. of Sci. and Technol., Daejeon, Korea, Republic of

Abstract: We have shown previously that strong neural signals for chosen value and choice outcome converge in the orbitofrontal cortex (OFC) in rats performing a dynamic foraging task. OFC neuronal activity was correlated with both bidirectional reward prediction error (RPE) and updated chosen value, suggesting local computation of RPE and value updating in the OFC. As a first step toward investigating neural circuit mechanisms of value computation in the OFC, we examined discharge patterns and stimulation effects of parvalbumin (PV)-expressing neurons in the mouse OFC. For this, we injected Cre-dependent virus carrying channelrhodopsin into the left or right OFC of PV-Cre mice, and trained them in a dynamic foraging task in a modified T-maze. We then implanted an optical fiber and an array of tetrodes in the OFC, and recorded unit signals with and without optical stimulation. We found that optogenetically-tagged PV neurons convey neural signals necessary to compute RPE, namely chosen value and choice outcome signals, when the outcome of the animal's choice was revealed. This finding suggests that PV-expressing neurons in the OFC participate in RPE computation and value updating. Unilateral optical stimulation during the reward period, during which RPE computation and value updating are thought to be accomplished, strongly suppressed discharges of optogenetically-untagged OFC neurons. However, optical stimulation had no effect on behavioral performance, and value-related neural activity in the subsequent behavioral stages was not significantly different between optically stimulated and unstimulated trials. These results indicate that locally suppressed value-related neural activity in the OFC can be readily recovered.

Disclosures: **Y. Jang:** None. **S. Jo:** None. **D. Kim:** None. **M. Jung:** None.

Poster

737. Neural Mechanisms of Value-Based Decision-Making

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Topic: H.01. Animal Cognition and Behavior

Support: Research Center Program of the Institute for Basic Science 329 (IBS-R002-G1)

Global PhD Fellowship, National Research Foundation of Korea (NRF)

Title: Responses of different neuron subtypes to expected value and trial outcome in medial prefrontal cortex

Authors: *D. KIM[1,3], H. JEONG[2,3], J. LEE[2], M. JUNG[1,2,3];
[1]Grad. Sch. of Med. Sci. and Engin., [2]Dept. of Biol. Sci., Korea Advanced Inst. of Sci. and Technol., Daejeon, Korea, Republic of; [3]Ctr. for Synaptic Brain Dysfunctions, Inst. for Basic Sci., Daejeon, Korea, Republic of

Abstract: Neuronal discharges in the prefrontal cortex (PFC) are strongly influenced by expected value and trial outcome. However, responses of different PFC neuron subtypes to these variables are not clearly understood. In this study, we monitored discharges of optogenetically identified parvalbumin (PV)- and somatostatin (SOM)-expressing neurons in the mouse prelimbic cortex during a probabilistic classical conditioning task. We found that PV and putative inhibitory interneurons (fast-spiking cells that were not optogenetically tagged) decrease and increase their firing rates following reward and punishment, respectively. By contrast, putative pyramidal neurons (regular-spiking cells that were not optogenetically tagged) were activated by reward and inhibited by punishment. SOM neurons, as a population, showed inconsistent responses to reward and punishment. During the cue and delay periods, SOM and putative pyramidal neurons showed stronger cue-specific responses than PV and putative inhibitory interneurons. In conclusion, PV neurons signaled both reward and punishment, with their response directions opposite from those of putative pyramidal cells, and SOM neurons and putative pyramidal neurons conveyed expected value signals.

Disclosures: **D. Kim:** None. **H. Jeong:** None. **J. Lee:** None. **M. Jung:** None.

Poster

737. Neural Mechanisms of Value-Based Decision-Making

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Topic: H.01. Animal Cognition and Behavior

Support: Institute for Basic Science (IBS-R002-G1)

Title: Effect of optogenetically stimulating mossy fiber terminals on spatial firing of CA3 neurons

Authors: *J. LEE[1,2], E. CHO[1], Y. KANG[1], J. LEE[1], M. JUNG[1,2];
[1]Ctr. For Synaptic Brain Dysfunctions, IBS, Daejon-City, Korea, Republic of; [2]Biol. Sci., Korea Advanced Inst. of Sci. and Technol., Daejeon, Korea, Republic of

Abstract: It is unclear whether and how discharges of dentate gyrus (DG) granule cells shape spatial firing of CA3 neurons in behaving animals. To investigate this matter, we injected Cre-dependent virus carrying a channelrhodopsin-2 variant (ChETA) to the dorsal DG of Rbp4-Cre mice that were trained to navigate a circular track to obtain water reward at two opposite locations. We then implanted an optical fiber and an array of tetrodes in CA3 region, and examined effects of optogenetically stimulating mossy fiber terminals on spatial firing of CA3 neurons. We found that some CA3 neurons could be reliably activated by optogenetic stimulation of mossy fiber terminals. As a consequence, their spatial firing on the maze was changed during optogenetic stimulation. Further, a subset of them maintained altered spatial firing even after turning off the stimulation. These results indicate that optogenetic stimulation of mossy fiber terminals can drive discharges and influence spatial firing of CA3 neurons in behaving animals. It is difficult to directly map our findings to effects of granule cell discharges on CA3 neuronal activity under natural conditions, because our stimulation is likely to activate a large number of mossy fiber terminals simultaneously. To address this issue, examining effects of inactivating DG granule cell inputs on CA3 neuronal activity is currently under way.

Disclosures: **J. Lee:** None. **E. Cho:** None. **Y. Kang:** None. **J. Lee:** None. **M. Jung:** None.

Poster

737. Neural Mechanisms of Value-Based Decision-Making

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Program#/Poster#: 737.07/HHH8

Topic: H.01. Animal Cognition and Behavior

Support: Research Center Program of Institute for Basic Science 329 (IBS-R002-G1)

Title: Spatial sequence-dependent discharges of hippocampal neurons

Authors: *B. BHATTARAI[1,2], J. W. LEE[2], M. W. JUNG[1,2];
[1]Korea Advanced Inst. of Sci. and Technol., Daejeon 34141, Korea, Republic of; [2]Ctr. for Synaptic Dysfunctions, Inst. for Basic Sci. (IBS), Daejeon 34141, Korea, Republic of

Abstract: In order to investigate hippocampal neural substrates of sequence memory, we recorded CA1 neuronal activity in rats performing a spatial sequence discrimination task. The maze contained a figure 8-shaped section through which the rats could navigate in one of four different spatial sequences (left-left, left-right, right-left and right-right) to obtain water reward. Each session consisted of four blocks of trials that were associated with four different correct sequences. The order of correct block sequences was pseudo-randomly determined, and each block consisted of a training (5 trials) and a test (15 trials) phase. We found three types of CA1 neural activity that conveyed sequence information. First, place fields in the common part of the maze were completely or partially remapped across different blocks. Second, neuronal population vector during the delay period (2-s time period before the onset of navigation through the figure 8-shaped section) changed across blocks. Third, a brief (~400 ms) and rapid replay of a particular place cell sequence during the period of immobility was found to be correlated to the place cell sequence in the current block more than expected by chance. Our results show that CA1 has multiple mechanisms for conveying spatial sequence information. It remains to be determined how they are related to remembering and generating spatial sequences.

Disclosures: B. Bhattarai: None. **J.W. Lee:** None. **M.W. Jung:** None.

Poster

737. Neural Mechanisms of Value-Based Decision-Making

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Topic: H.01. Animal Cognition and Behavior

Support: Institute for Basic Science 329 (IBS-R002-G1)

Global PhD Fellowship, National Research Foundation of Korea (NRF)

Title: Inactivation of dorsal CA1, but not dorsal CA3, impairs value learning in a dynamic foraging task

Authors: *Y. JEONG[1,2], J. LEE[1,2], J. LEE[2], M. JUNG[1,2];
[1]Dept. of Biol. Sci., KAIST, Daejeon, Korea, Republic of; [2]Ctr. for Synaptic Brain Dysfunctions, Inst. for Basic Sci., Daejeon, Korea, Republic of

Abstract: We have shown previously that dorsal CA1 of the rat hippocampus conveys robust value signals. Here we examined behavioral effects of inactivating dorsal CA1 and CA3 on value-based decision making. We injected Cre-dependent *hM4Di* virus bilaterally into dorsal CA1 and CA3 of Camk2a-Cre and Grik4-Cre mice, respectively, and trained them in a dynamic two-armed bandit task. The mice were then tested in the task following clozapine-N-oxide (CNO, 5mg/kg, i.p.) or vehicle (DMSO) injection. A reinforcement learning model-based analysis revealed that learning rate (α) was slightly, but significantly lower in CNO- compared to vehicle-injected Camk2a-Cre mice. However, the randomness in action selection (β) was not significantly different between the two animal groups. Consistent with these results, the proportion of rewarded trials was significantly lower in CNO- compared to vehicle-injected Camk2a-Cre mice during the dynamic state (early trials after changing reward probabilities), but not during the steady state (late trials after changing reward probabilities). No significant effect of CNO injection was found whatsoever in Grik4-Cre mice. These results show that inactivation of dorsal CA1, but not CA3, impairs value learning without influencing value-dependent action selection. Our results suggest the involvement of dorsal CA1 not only in episodic learning, but also in incremental value learning.

Disclosures: **Y. Jeong:** None. **J. Lee:** None. **J. Lee:** None. **M. Jung:** None.

Poster

737. Neural Mechanisms of Value-Based Decision-Making

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Topic: H.01. Animal Cognition and Behavior

Support: T32 ES007051

Title: Assessment of working memory and monoamine neurotransmitters in methamphetamine-treated adult male rats

Authors: *A. GUTIERREZ[1,3], C. V. VORHEES[2,4], M. T. WILLIAMS[2,4];
[1]Neurosci. Grad. Program, [2]Div. of Neurol., Cincinnati Children's Hosp. Med. Ctr., Cincinnati, OH; [3]Col. of Med., [4]Dept. of Pediatric Neurol., Univ. of Cincinnati, Cincinnati, OH

Abstract: Chronic methamphetamine (MA) abuse produces long-term cognitive deficits on learning and memory (L&M). We have demonstrated that allocentric L&M in rats is disrupted by treatment with a neurotoxic regimen of MA as assessed using the Morris Water Maze (MWM). Human studies also demonstrate that long-term MA usage negatively impacts working memory. Rodent models, however, have only demonstrated subtle or transient effects on working memory. The aim of this study was to examine whether a neurotoxic binge MA model (4 x 10 mg/kg at 2 h intervals) in adult male Sprague-Dawley rats would result in deficits in working memory. Two separate experiments were conducted. Rats were treated with MA or Saline and allowed to recover for 2 weeks. In the first experiment, a MWM matching-to-sample (MTS) procedure was used with one sample trial and two recall trials given each day for 6 days. The first recall trial occurred immediately after the sample trial, while the second recall trial occurred 30 min after the sample trial. In the second experiment, brain tissue was collected for analysis of monoamines 2 weeks after treatment. Monoamines were analyzed by HPLC-ECD. In the MTS task, a significant effect of MA was detected at the time point immediately following the sample trial, i.e., animals treated with MA were negatively affected in their ability to find the platform compared with controls. Animals treated with MA also performed worse than controls at the 30 min time point, although this only approached significance. Further analysis revealed that animals treated with MA had more difficulty switching strategies as evidenced by spending significantly more time in quadrants where the platform resided the previous day. Analysis of monoamines showed significantly decreased levels of dopamine and serotonin in the dorsal striatum and nucleus accumbens. Serotonin levels were also significantly decreased in the hippocampus. These data provide a foundation to examine the mechanism underlying working memory deficits following MA neurotoxicity.

Disclosures: **A. Gutierrez:** None. **C.V. Vorhees:** None. **M.T. Williams:** None.

Poster

737. Neural Mechanisms of Value-Based Decision-Making

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Program#/Poster#: 737.10/HHH11

Topic: H.01. Animal Cognition and Behavior

Support: Grant of University of shahid beheshti

Title: Functional Interaction between Orexin 2 and CB1 receptors within the ventral tegmental area in the lateral hypothalamic-induced place preference in the rats

Authors: *A. KASHEFI[1], M. MORADI[2], A. HAGHPARAST[2], M. ZABIHI[3];
[1]Univ. De Brasilia, Brasilia, Brazil; [2]Neurosci., Shahid Beheshti Univ. of Med. Sciences, Tehran, Iran, Tehran, Iran, Islamic Republic of; [3]Islamic Azad Univ. of Semnan, Semnan, Iran, Islamic Republic of

Abstract: Lateral hypothalamus (LH) orexinergic system has direct connections with the ventral tegmental area (VTA) and this areas have a key role in the acquisition of conditioned place preference (CPP) induced by chemical stimulation of the LH. Nevertheless, it remains unknown what is the function of two kinds of orexin receptors and the interaction between the orexinergic and cannabinoid systems in the reward circuit. In this study, we tried to clarify the involvement of orexin-2 (OX2) and CB1 receptors within the VTA in development of CPP after chemical stimulation of the LH by carbachol. Rats were implanted by two separate cannulae into the LH and VTA, unilaterally. The CPP paradigm was done; conditioning scores and locomotor activities were recorded by Ethovision system. Results showed that administration of TCS OX2 29 as a selective OX2 receptor antagonist (1, 3 and 10 nM/0.3 µl DMSO) and AM251 as a selective CB1 receptor antagonist (5, 25 and 125 µM/0.3 µl DMSO) into the VTA just 5 min prior to microinjection of carbachol (250 nM/0.5 µl saline) into the LH during the 3-day conditioning phase, could inhibit the development of CPP. On the other hand, concurrent injection of effective doses of TCS OX2 29 and AM251 into the VTA could reduce conditioning scores significantly. In addition, ineffective doses of both antagonists into the VTA concurrently did not have any effects on the LH-induced CPP. Our findings showed that OX2 and CB1 receptors have a critical role in modulating the reward circuit in the VTA. Moreover, results of this study suggest that cannabinoid and orexinergic systems within the VTA act through the same post receptor mechanism to show the rewarding effects of LH stimulation in the rats.

Disclosures: **A. Kashefi:** None. **M. Moradi:** None. **A. Haghparast:** None. **M. Zabihi:** None.

Poster

737. Neural Mechanisms of Value-Based Decision-Making

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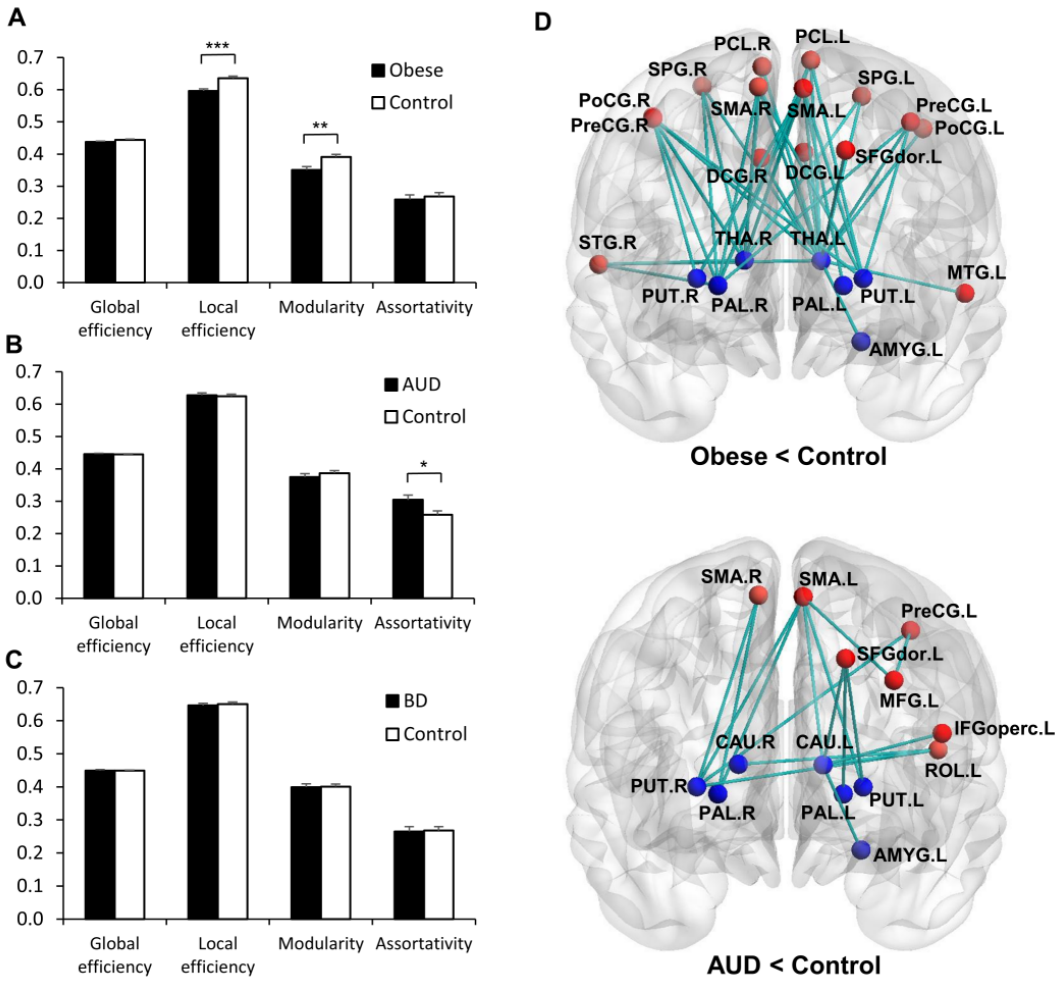
Topic: I.06. Computation, Modeling, and Simulation

Support: Wellcome Trust Fellowship grant (093705/Z/10/Z)

Title: Disrupted brain networks in obesity and alcohol use disorder: a data-driven graph theory approach

Authors: *K. BAEK[1,2], P. KUNDU[3], V. VOON[2];
[1]UNIST, Ulsan, Korea, Republic of; [2]Dept. of Psychiatry, Univ. of Cambridge, Cambridge, United Kingdom; [3]Departments of Radiology and Psychiatry, Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: The efficient organization and communication of brain networks underlies cognitive processing and pathological behaviors. However, few studies have focused on whole-brain networks in the maladaptive consumption behaviors such as obesity and alcohol use disorders. Here we use a novel multi-echo resting state functional MRI technique along with a data-driven graph theory approach to assess global and regional network characteristics in obesity, alcohol use disorder (AUD) and binge drinking (BD). Multi-echo resting state functional MRI scans were taken from 40 obese subjects, 39 AUD subjects, 61 BD subjects and their matched healthy control groups. The normalized correlation across 90 regions of interest (ROI) from the Automated Anatomical Labeling template was estimated, and global and regional network properties were computed in the binarized networks. Group difference in region-to-region connectivity was assessed using Network Based Statistics. We show that obese subjects have significantly reduced local efficiency and modularity exhibiting disrupted local clustering and small-world network properties. Obese subjects also showed decreased connectivity of cortico-striatal- thalamo-cortical networks related with motor function along with increased nodal degree in visual cortices. Subjects with alcohol use disorders showed significantly increased network assortativity along with decreased connectivity of both motor and prefrontal cortico-striatal networks. That subjects with binge drinking lacked significant differences from healthy volunteers suggests a role for chronic alcohol exposure in mediating these findings. Using a data-driven graph theory analysis approach, we highlight impairments in cortico-striatal networks across both obesity and alcohol dependence. These findings are consistent with theories of habit formation implicating a shift towards putaminal regions. Alterations in brain networks may act as potential biomarkers and therapeutic targets in disorders of addiction.



Disclosures: **K. Baek:** None. **P. Kundu:** None. **V. Voon:** None.

Poster

738. Memory Consolidation and Reconsolidation: Fear Conditioning Circuits

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 738.01/HHH13

Topic: H.01. Animal Cognition and Behavior

Title: Trace fear conditioning can affect previously acquired context memories.

Authors: ***D. S. REIS**, E. K. ROTONDO, F. J. HELMSTETTER;
Univ. of Wisconsin - Milwaukee, Milwaukee, WI

Abstract: It is generally accepted that prior experience can have a significant impact on subsequent learning. This can be seen in work on fear generalization in which prior fear conditioning can induce generalized fear responses to stimuli that were never explicitly paired with an aversive stimulus but are similar, in various ways, to the paired stimuli. How prior experience can influence exactly what is being learned during subsequent tasks remains less clear. We compared the effects of training with trace fear conditioning (TFC), in which the pairing of an auditory conditioned stimulus (CS) with an aversive foot shock (UCS) is separated by a brief stimulus free period, or explicitly unpaired presentations of the CS and UCS, on the behavioral expression of previously acquired contextual memories. On day 1 (L1) in context A (CXT A), animals were given 1 of 4 different experiences. They either remained in the home cage (HC), or received an unsignaled foot shock immediately upon being placed into CXT A (IMM), shock after a 180s delay (CFC), or not at all (CE). These treatments allowed us to delineate the effects of a second learning experience on groups of animals that learned different things about CXT A. Rats were given TFC or unpaired CS/UCS presentations as a second learning procedure (L2) in a novel context (CXT B) 24 hours after L1. Memory for the L1 training experience was tested in CXT A 48 hrs after L2 training. Previous work has shown that time spent in the training context prior to foot shock presentation is required for the formation of a context fear memory. For example, foot shock presentation 180s after being placed in the training context but not when presented immediately results in robust context freezing 24 hrs later. Our results support this immediate shock deficit but only when rats received unpaired CS/UCS presentations as L2. In comparison, when given TFC as L2, rats given immediate shock at L1 showed fear responses when tested in CXT A. Interestingly, no differences were found in CXT A freezing for the context exposure (CE) group, regardless of L2 treatment suggesting that, in general, the way in which later learning can influence the expression of prior context memories may depend on what was learned during those previous experiences. Current work is investigating the contributions of the amygdala, hippocampus and prefrontal cortex in mediating this effect.

Disclosures: **D.S. Reis:** None. **E.K. Rotondo:** None. **F.J. Helmstetter:** None.

Poster

738. Memory Consolidation and Reconsolidation: Fear Conditioning Circuits

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Program#/Poster#: 738.02/HHH14

Topic: H.01. Animal Cognition and Behavior

Support: NIMH R01 MH069558

Title: Exploring contributions of stimulus modality and stimulus-stimulus contingencies to the cortical organization of remote fear memory

Authors: *E. K. ROTONDO, D. S. REIS, N. C. FERRARA, J. C. CICHON, F. J. HELMSTETTER;
Psychology, Univ. of Wisconsin- Milwaukee, Milwaukee, WI

Abstract: Long-term memories may undergo *systems consolidation*, a gradual transition to distributed neocortical memory storage that develops through time-dependent changes in cortico-cortical functional connectivity. How the specific information encoded at the time of learning shapes the organization of this remote form of memory in the brain is not well understood. Here we begin to examine modality-specific and cross-modal involvement of secondary sensory cortices in remote fear memories by manipulating the modality of and predictive relationship between multiple sensory cues at the time of learning. Specifically, using second-order fear conditioning with discrete auditory and visual cues, learning-dependent neural plasticity in secondary auditory (AuV/TeA) and visual (V2L) cortices was examined. Time-dependent increases in the expression of the synaptic marker synaptophysin were observed as a function of conditioning with auditory and visual stimuli in AuV/TeA and V2L, respectively, which is consistent with the idea that patterned changes in cortical synaptic connectivity reflect the specific content of stored information. Functionally**,** remote retrieval of either the first-order auditory conditional stimulus or the second-order visual conditional stimulus was associated with significantly greater expression of the neuronal activity marker zif268 in AuV/TeA relative to recent retrieval controls. These data suggest that secondary sensory cortices may store modality-specific information at remote time points, but may also exhibit cross-modal recruitment at the time of retrieval if the initial learning involves the association of multiple cues. Together, these results support an encouraging new approach to understanding the organizational principles of neocortical memory storage.

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Poster

738. Memory Consolidation and Reconsolidation: Fear Conditioning Circuits

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Topic: H.01. Animal Cognition and Behavior

Support: NIMH R01 MH069558

Title: Medial geniculate nucleus input modulates amygdala encoding during fear memory discrimination

Authors: *N. FERRARA, P. K. CULLEN, E. K. ROTONDO, F. J. HELMSTETTER; Psychology, Univ. of Wisconsin--Milwaukee, Milwaukee, WI

Abstract: The generalization of fear is characterized by abnormal fear responding to safety cues and elevated amygdala activity, and is common in individuals diagnosed with post-traumatic stress disorder. Differential fear conditioning provides a way to measure learned fear responses, as well as the factors contributing to the generalization of fear. During conditioning, two auditory cues are presented where one is consistently paired with a foot shock (CS+) and one is never paired with shock (CS-). Differential responding between cues demonstrates discrimination, while heightened responding to the CS- is considered generalization of fear. Discrimination learning depends on a network of brain structures, with a majority of the work focusing on elevated amygdala activity as a critical component underlying generalization. Synapses in the amygdala, representing information from auditory cortex (ACx) and medial thalamus (MgN), undergo plastic changes in response to fear conditioning and are major contributors to the degree of fear expression to both CS+ and CS-. Heightened ACx or MgN presynaptic input contributes to elevated amygdala activity underlying fear generalization. However, the contribution of MgN and ACx inputs to the amygdala leading to generalization remain unclear. Recent work illustrates that ACx activity may not be the primary factor underlying elevated responding to safety cues, while the MgN has been implicated in the encoding of a differential fear memory and generalization of auditory cues. The effects of MgN inhibition on amygdala activity as well as the molecular mechanisms underlying MgN-dependent fear memory discrimination and generalization have not been directly tested. Persistent fear responding is mirrored by surface expression of amygdala AMPA receptors, which can serve as molecular markers for fear memory formation. Amygdala AMPA receptor are immediately post synaptic to MgN terminals, providing an approach to measure thalamo-amygdala synaptic modifications related to fear learning. Here we characterize the role of the MgN in supporting differential fear memory formation and expression and looked at molecular mechanisms underlying increases in fear expression to CS-. We found that protein synthesis dependent plasticity in the MgN is critical for the consolidation of a differential fear memory as well as the update from low to high generalization of fear. Furthermore, MgN protein synthesis modulates amygdala synaptic scaffolding and AMPA receptor expression underlying fear memory formation.

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Poster

738. Memory Consolidation and Reconsolidation: Fear Conditioning Circuits

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Topic: H.01. Animal Cognition and Behavior

Support: NIMHRO1MH069558

Title: Behavioral expression of a fear memory is maintained by neural activity in a distributed brain network throughout CS presentation

Authors: *P. K. CULLEN[1], N. C. FERRARA[2], S. E. PULLINS[2], J. M. HINTZ[2], F. J. HELMSTETTER[1];
[1]Psychology, Univ. of Wisconsin, Milwaukee, Milwaukee, WI; [2]Univ. o f Wisconsin, Milwaukee, Milwaukee, WI

Abstract: It is well accepted that the amygdala is required for the acquisition, retrieval and expression of a conditional fear memory. For instance, presentation of the conditional stimulus (CS) elicits neural spiking in the lateral amygdala (LA) that correlates with fear acquisition/expression. However, behavioral expression of fear persists well beyond bursts of LA activity, suggesting that LA activity does not maintain behavioral fear responses during the entire CS period. Recent evidence suggests that activity of the prelimbic cortex (PL) is required for conditional fear expression and that PL unit activity persists throughout presentation of the CS at retrieval. Fear expression also depends on activity in both the medial central nucleus of the amygdala (CeM) and the ventrolateral part of the periaqueductal gray (vlPAG). However, our present understanding of LA, PL, CeM, and vlPAG function comes primarily from electrophysiological recordings and pharmacological manipulations or lesions that render the structure(s) impaired for an imprecise duration of time following drug administration. To understand the specific time-dependent role of the LA-PL-CeM-vlPAG circuit during memory retrieval, activity within each of these regions was optogenetically inhibited using virally-mediated expression of the light-driven proton pump ArchT (AAV-CAG-ArchT-GFP) in a temporally precise manner during auditory CS presentations. Animals were trained with auditory fear conditioning and exposed to two 30-second white noise CS presentations during a retrieval session. In one condition, laser stimulation occurred simultaneously with the CS (100% temporal overlap). In a second condition, animals received an equal amount of laser stimulation that was shifted starting 15-seconds after CS onset (50% temporal overlap). We found that: 1) inactivation of LA only impaired CS freezing in the 100% overlap condition; 2) inactivation of the PL, CeM and vlPAG impaired CS freezing in both the 100% and 50% overlap conditions; 3) inactivation of ArchT-containing CeM inputs terminating in the vlPAG during CS presentation impaired freezing. These results indicate that fear expression is triggered by initial LA activity and

maintained by persistent neuronal firing of the PL, CeM and vlPAG. By achieving within-CS temporal resolution of neural inhibition, the current study provides new insight into the neural network responsible for maintaining behavioral control during memory retrieval. A deeper understanding of how fear is maintained through a distributed neural network will provide new therapeutic targets for aberrant fear memory expression exhibited in many psychiatric disorders.

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Poster

738. Memory Consolidation and Reconsolidation: Fear Conditioning Circuits

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Topic: H.01. Animal Cognition and Behavior

Support: NIMH R01MH069558

Title: Activity-dependent protein degradation and age-related memory impairment.

Authors: *S. E. PULLINS, P. K. CULLEN, N. C. FERRARA, J. R. MOYER, Jr., F. J. HELMSTETTER;
Psychology, Univ. of Wisconsin - Milwaukee, Milwaukee, WI

Abstract: Humans and rats exhibit deficits in episodic/explicit memories with normal aging. Recent research implicates ubiquitin proteasome system (UPS)-mediated protein degradation as a key factor in the synaptic plasticity supporting memory formation and retrieval. In rats, normal aging leads to decreased basal proteolytic activity in several brain structures known to support acquisition and retrieval of trace fear conditioning (TFC) memory. Using adult (3 months), middle-aged (15 months), and aged (22 months) F344 rats, we show that UPS-mediated protein degradation in the amygdala and prefrontal cortex is decreased in aged rats following TFC memory retrieval, as evidenced by reduced phosphorylation of RPT6, a known regulator of 26S proteasome activation. Additionally we show decreased clearance of polyubiquitinated protein in the amygdala of aged rats following retrieval. Compared to young adult animals, aged rats also displayed altered levels of a known target of the AMPA receptor associated synaptic scaffolding protein SHANK – a UPS target – following retrieval. Finally, we investigated whether impairing proteolysis in the amygdala of young adult rats would lead to a deficit in TFC. Pre-training infusions of the proteasome inhibitor clasto-Lactacystin ß-lactone into the amygdala of adult rats significantly impaired trace fear memory when tested 24 hours following acquisition. These data suggest that the deficit in TFC observed in aged rats may be due, in part, to a decrease in

plasticity-dependent protein degradation. These experiments augment the growing body of literature indicating that stimulation of the proteasome could offer a novel means of treating cognitive decline during normal aging.

Disclosures: S.E. Pullins: None. **P.K. Cullen:** None. **N.C. Ferrara:** None. **J.R. Moyer:** None. **F.J. Helmstetter:** None.

Poster

738. Memory Consolidation and Reconsolidation: Fear Conditioning Circuits

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Topic: H.01. Animal Cognition and Behavior

Support: MH069558

Title: Activity-dependent protein degradation and GluR2 endocytosis in the amygdala regulate reconsolidation-dependent reevaluation of a contextual fear memory

Authors: N. C. FERRARA[1], T. J. JAROME, 53201[1], P. K. CULLEN[1], J. L. KWAPIS[1], *F. J. HELMSTETTER[2];
[1]Univ. of Wisconsin-Milwaukee, Milwaukee, WI; [2]Deptartment of Psychology, Univ. of Wisconsin Milwaukee Dept. of Psychology, Milwaukee, WI

Abstract: Memories for associations learned during Pavlovian fear conditioning are rapidly acquired, robust and long lasting and provide an ideal model for studying long-term fear memory formation and storage. During training, a conditional stimulus (CS) acquires aversive value through pairings with an unconditional stimulus (UCS), and memory for this association is stored through a protein-synthesis dependent consolidation. During retrieval, re-exposure to the CS renders the memory temporarily labile and new protein synthesis is necessary for the transfer of the memory back into long-term storage, a process called reconsolidation. Reconsolidation is thought to occur each time a memory is retrieved, providing a potential means of intervention to disrupt or modify established fear memories. The reconsolidation process is dependent on protein degradation, *de novo* protein synthesis, and GluR2-AMPA receptor trafficking in the amygdala and occurs in the presence of new information during the retrieval session (e.g., Jarome et al., 2015). Interestingly, the association learned during fear conditioning can be updated or "reevaluated" during retrieval, allowing for strengthening or weakening of the original memory. Very little is currently known about the mechanisms responsible for modification during retrieval and how it relates to reconsolidation. Here we characterized behavioral and molecular mechanisms underlying contextual fear memory updating in rodents by

modifying the memory during a brief retrieval session. We trained rats to associate a novel context (CS) with a foot shock (UCS) and the following day changed the value of the UCS by weakening the shock intensity. Surprisingly, we found the presentation of two foot shocks of weaker intensity during retrieval heightened fear responding on a later test, suggesting that the addition of new information during retrieval modifies the original aversive value which is reflected in elevated fear expression. Furthermore, we found that inhibiting proteasome activity or GluR2 endocytosis in the amygdala prevented memory strengthening, suggesting that updating was likely occurring through reconsolidation of the original memory trace. These results may have important therapeutic implications for memories for traumatic events that are resistant to modification. In future experiments, we plan to determine if destabilization occurs in the same cells that are activated during retrieval dependent reevaluation.

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Poster

738. Memory Consolidation and Reconsolidation: Fear Conditioning Circuits

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Title: Autophagy induction enhances memory destabilization beyond reconsolidation boundary

Authors: *M. H. SHEHATA[1,3], Q. ZHAO[1,3], K. ABDOU[1,3], M. MATSUO[2], H. NISHIZONO[2,3], K. INOKUCHI[1,3];
[1]Dept. of Biochem., [2]Div. of Animal Exptl. Lab., Univ. of Toyama, Toyama, Japan; [3]Japan Sci. and Technol. Agency (JST), CREST, Kawaguchi, Japan

Abstract: There is a wide interest in memory reconsolidation as a target to treat pathogenic memories; despite, mechanistically vague boundary conditions have been described that constrain the likelihood of a memory from being destabilized after recall. It has been reported that inhibiting synaptic protein degradation prevents memory destabilization. However, whether the reverse relation is true and whether it can be utilized to overcome these boundary conditions is still unknown. Here, we show that induction of autophagic protein degradation enhances synaptic destabilization in a long-term potentiation (LTP)-reconsolidation model in freely moving rats, and enhances fear memory destabilization in a contextual fear reconsolidation paradigm in mice. Furthermore, autophagy induction overcomes a boundary condition for auditory fear memory reconsolidation. The autophagy induction effects are dependent on AMPA receptor endocytosis and correlates with its degradation in ensemble neurons. Our results complement the evidence for a causal relationship between protein degradation and memory destabilization, give more insight on the mechanisms of memory destabilization and the boundary conditions, and suggest autophagy inducers as a useful tool for the treatment of memory-related psychological diseases such as post-traumatic stress disorder (PTSD).

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Poster

738. Memory Consolidation and Reconsolidation: Fear Conditioning Circuits

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Topic: H.01. Animal Cognition and Behavior

Support: KAKENHI (23220009) and (25115002)

Title: Adult neurogenesis in maintenance of the hippocampal learning capacity

Authors: *M. ALAM[1,3], T. KITAMURA[4], Y. SAITOH[1,3], N. OHKAWA[1,3], T. KONDO[2], K. INOKUCHI[1,3];
[1]Dept. of Biochem., [2]Dept. of Radiological Sci., Univ. of Toyama, Toyama-Shi, Japan; [3]CREST, JST, Toyama, Japan; [4]RIKEN-MIT Ctr. for Neural Circuit Genetics, MIT, Cambridge, MA

Abstract: Saturation of the hippocampal long-term potentiation (LTP), a cellular basis of learning and memory, impairs hippocampus-dependent learning. This impairment recovers in parallel with the LTP decay. However, the driving factor that regulates the recovery of learning capacity remains unclear. Neurogenesis occurs in the brain of adult mammals throughout life and is involved in the decay process of hippocampus-dependency of memories. Here we show that adult neurogenesis plays a crucial role in the maintenance of hippocampal capacity for learning. Delivery of repeated high frequency tetanic stimulation (rHFS) to perforant pathway saturated the dentate gyrus LTP and impaired the learning capacity in contextual fear conditioning (CFC), a hippocampus-dependent learning task, whereas it had no effect on the hippocampus-independent learning. The impaired learning capacity completely recovered after 14 days when LTP decayed. Ablation of neurogenesis by X-ray irradiation retained the hippocampal LTP and delayed the recovery of learning capacity from the saturation. Similar results were obtained when repeated maximum electroconvulsive shock was employed to saturate the hippocampal LTP. Moreover, enhancing neurogenesis by running wheel speeded up the decay of LTP from the rHFS-induced LTP saturation and exhibited an earlier recovery of learning in the CFC task. These results indicate that the learning capacity gradually recovered in parallel with the gradual decay of LTP and decreased neurogenesis delayed the recovery of learning by retaining the rHFS-evoked LTP level whereas increased neurogenesis accelerates the recovery process. Our findings unravel a new role for neurogenesis, suggesting that adult neurogenesis is crucial for renewal of the hippocampal memory circuits.

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Poster

738. Memory Consolidation and Reconsolidation: Fear Conditioning Circuits

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Topic: H.01. Animal Cognition and Behavior

Support: JSPS KAKENHI 23220009

CREST, JST

MEXT KAKENHI 25115002

Title: Neuronal ensemble orchestrated intersection of two distinct emotional memory traces

Authors: *J. YOKOSE[1,4], R. OKUBO-SUZUKI[1,4], M. NOMOTO[1,4], N. OHKAWA[1,4], H. NISHIZONO[2,4], M. MATUSO[2], A. SUZUKI[1,4], Y. TAKAHASHI[5], M. NAGASE[5], A. M. WATABE[5], M. SASAHARA[3], F. KATO[5], K. INOKUCHI[1,4];
[1]Dept. of Biochem. Grad. Sch. of Med. & Pharmaceut. Sci., [2]Div. of Animal Exptl. Laboratory, Life Sci. Res. Ctr., [3]Dept. of Pathology, Univ. of Toyama, Toyama, Japan; [4]CREST,Japan Sci. and Technol. Agency, Tokyo, Japan; [5]Dept. of Neurosci., Jikei Univ. Sch. of Med., Tokyo, Japan

Abstract: Memory retrieval process is imbued with multisensory cues such as sights, sounds, smells and tastes, which lead to its prior memories and emotions. These distinct units of information that have been stored in the brain are sometimes reactivated excessively and interact to generate a new associative memory, which can leads to psychiatric disorders. Previous studies suggested that retrieval-induced reactivation of emotional memory plays a potential role in the interaction with pre-existing memory.
However, it has been uncertain at the level of neuronal ensemble how two distinct memories are reorganized to generate and integrate an associative memory.
Here, to address this issue, we conceived of the combination of two amygdala-dependent behavioral paradigms, conditioned taste aversion task (CTA) and auditory cued fear conditioning (AFC). Mice were trained in CTA and AFC independently, where animals did not associate these two paradigms. After each memory was formed, animals received, in the reactivation session, synergistic and repetitive presentations of the condition stimuli (CS) for CTA and AFC, which induced the retrieval of both associative memories at the same time. We found that presenting the CS of CTA triggered the condition response of AFC such as freezing-like behavior, suggesting that the reactivation session leads to the interaction between CTA and AFC memories. After undergoing both CTA and AFC retrievals, Arc/Homer1a catFISH analysis elucidated an increase in the ratio of co-sharing neuronal subpopulation (overlapping ensemble) in the basolateral amygdala (BLA). We then optogenetically silenced AFC ensemble in BLA, which was specifically activated in the conditioning, during the reactivation session by applying the tet-OFF lentiviral vector encoding archaerhodopsin (ArchT) to the c-fos tTA transgenic mice. Suppression of the AFC ensemble activity impaired the freezing-like behavior that was, in control case, triggered by presenting the CS of CTA and decreased the proportion of the overlapping ensemble between the CTA-retrieval and AFC-retrieval ensembles. We also found that optogenetic silencing the activity of the overlapping ensemble, which was generated by repeated activation during AFC and CTA retrieval, was sufficient to reduce the freezing-like behavior. Importantly, original memories in both CTA and AFC were intact through the whole test sessions but only the linkage between CTA and AFC memories was interrupted.
Taken together, our findings provide new insight into a specific neuronal ensemble shared with two independent memories, which was generated through multiple reactivations, subserve an associative memory.

Disclosures: J. Yokose: None. **R. Okubo-suzuki:** None. **M. Nomoto:** None. **N. Ohkawa:** None. **H. Nishizono:** None. **M. Matuso:** None. **A. Suzuki:** None. **Y. Takahashi:** None. **M. Nagase:** None. **A.M. Watabe:** None. **M. Sasahara:** None. **F. Kato:** None. **K. Inokuchi:** None.

Poster

738. Memory Consolidation and Reconsolidation: Fear Conditioning Circuits

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Title: Optical manipulation of parietal association cortex regulates contextual fear memory

Authors: *A. SUZUKI[1,2], S. KOSUGI-USHIJIMA[1,2], N. OHKAWA[1,2], M. MATSUO[3], H. NISHIZONO[3,2], K. INOKUCHI[1,2];
[1]Dept. of Biochemistry, Grad. Sch. of Med. & Pharmaceut. Sci., Univ. of Toyama, Toyama, Japan; [2]CREST, Japan Sci. and Technol. Agency, Tokyo, Japan; [3]Div. of Animal Exptl. Laboratory, Life Sci. Res. Center, Univ. of Toyama, toyama, Japan

Abstract: In the usual contextual fear conditioning, the animals have two types of learning [(1) learning the context (CS) and, (2) learning the association between the context (CS) and the shock (US)]. Because these types of learning co-occur during conditioning, it is difficult to identify brain regions required for CS-US association. In this study, we modified behavioral paradigm, context-pre-exposure facilitation effect (CPFE) paradigm, in which the mice received paired or unpaired presentations of the CS and US during conditioning. Using this behavioral paradigm, we performed Arc CatFISH method to detect brain regions required for the CS-US association and found that parietal association cortex (PtA) responded to CS-US signals. To elucidate roles of PtA in CS-US association, we examined whether manipulating the PtA activity by optogenetics technique generate artificial CS-US associative memory. By employing lentivirus harboring 3GTRE-ChR2-EYFP and cfos-tTA transgenic mice, we labeled a subset of PtA neurons that responded to CS context. Optical stimulation of these PtA neurons, when mice received footshock in a different context, generated a false memory, in which mice showed a freezing response in the initial context where mice did not received footshock. These finding

suggest that optical stimulation of the PtA cellular ensemble is capable of generating an artificial CS-US associative memory. Furthermore, we asked whether manipulating the PtA activity by optogenetics technique regulates CS-US associate memory that has been once formed. We labeled with ArchT-EYFP a subset of PtA neurons of cfos-tTA transgenic mice that responded during reactivation. Optically silencing these PtA neurons, when mice were exposed to CS context 24h after reactivation, suppressed associative memory. However when mice were again tested 24h later, mice showed high freezing, indicating that PtA regulates memory retrieval. On the other hand, 15min optical silencing immediately after CS exposure 24h after reactivation suppressed fear memory when mice were tested 24h later without optical silencing, indicating that manipulating the PtA activity by optogenetics technique erase CS-US associative memory. From these findings we concluded that optical manipulation of PtA activity regulates contextual fear memory.

Disclosures: **A. Suzuki:** None. **S. Kosugi-Ushijima:** None. **N. Ohkawa:** None. **M. Matsuo:** None. **H. Nishizono:** None. **K. Inokuchi:** None.

Poster

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Topic: H.01. Animal Cognition and Behavior

Support: RO1 NS088053 to B.J.W.

Title: DREADD-mediated inhibition of hippocampal neurons during memory retrieval and consolidation

Authors: ***J. N. KRUEGER**[1], A. P. CRESTANI[3], L. M. STRAEHLE[1], B. J. WILTGEN[1,2]; [1]Ctr. for Neurosci., [2]Dept. of Psychology, Univ. of California Davis, Davis, CA; [3]Neurosci. Grad. Program, Federal Univ. of Rio Grande do Sul, Porto Alegre, Brazil

Abstract: The current experiments examined the effects of DREADD-mediated inactivation of hippocampal neurons on memory retrieval and consolidation. To do this, we expressed the inhibitory DREADD hM4di (driven by the synapsin1 or CaMKII promoter) in the dorsal hippocampus of wild-type mice. Two weeks after AAV infusions, animals were trained in contextual fear conditioning. For the retrieval experiments, animals were tested one hour after receiving an IP injection of either CNO or DMSO. For the consolidation experiments, animals were given water bottles containing either CNO or DMSO 1 day after training. Ten days later, they were tested. Freezing was used as an index of memory and expression of the immediate-

early gene (IEG) c-Fos was used as a measure of neuronal activation. As expected, Syn-Hm4Di animals treated with CNO showed impaired memory retrieval and consolidation. Unexpectedly, these same animals showed significantly higher levels of c-Fos expression (3x) than their DMSO-treated counterparts. Our results are consistent with a recent paper that demonstrated promoter-specific effects of Hm4Di inhibition in the dorsal hippocampus (A. Lopez et. al, 2016). In this paper (and in our experiment) the behavioral effects of synapsin1-promoted Hm4Di are likely due to silencing of inhibitory neurons, which then increases the activity of pyramidal cells (A. Lopez et. al, 2016). In our CaMKII-Hm4Di experiment, we found that animals receiving CNO did not exhibit impaired memory retrieval despite showing significantly lower levels of c-Fos expression than their DMSO-treated counterparts. This lack of an effect may reflect compensation by other brain regions at the time of testing as has been reported by other groups when prolonged silencing methods are used (Goshen et. al., 2011) Studies are currently being conducted to determine if CaMKII-hM4Di mediated silencing impairs memory consolidation.

Disclosures: **J.N. Krueger:** None. **A.P. Crestani:** None. **L.M. Straehle:** None. **B.J. Wiltgen:** None.

Poster

738. Memory Consolidation and Reconsolidation: Fear Conditioning Circuits

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Topic: H.01. Animal Cognition and Behavior

Support: RO1 NS088053 to B.J.W.

NSF GRFP to A.B.H

Title: Interactions between the hippocampus and cortex during remote memory retrieval

Authors: ***A. HAMIDI**[1], Y. OTA[2], D. MARDINI[2], S. L. SUAREZ[2], B. J. WILTGEN[3]; [1]Univ. of California Davis, Davis, CA; [3]Dept. of Psychology and Ctr. for Neurosci., [2]Univ. of California, Davis, Davis, CA

Abstract: According to the theory of systems consolidation, the brain circuits required to successfully retrieve a memory depends on the age of the memory itself. Specifically, the theory claims that newer (recent) memories are hippocampal dependent while older (remote) memories are hippocampal independent. The claim that the hippocampus plays a temporally-limited role in memory retrieval is based partly on data from patients with hippocampal/medial temporal lobe

(HPC/MTL) damage who exhibit temporally-graded retrograde amnesia. This observation led to the idea that, over time, memories undergo a stabilization process (i.e. consolidation) which transforms or transfers the initial representation from the hippocampus to cortical networks. This transfer is thought to depend upon the hippocampus reinstantiating specific patterns of neuronal activity in corresponding cortical networks. Our group recently provided direct evidence (Tanaka et al., 2014) that reactivation of a specific ensemble of hippocampal neurons is necessary (1) for the successful retrieval of a recently formed memory and (2) for the normal reactivation of cortical (entorhinal, retrosplenial, and perirhinal) ensembles. Using similar methods, the current study aims to extend these findings by testing the time-dependent involvement of the hippocampus at remote time points. To do this, we performed bilateral infusions of a viral construct containing a double-inverted floxed version of the light-sensitive proton pump, archearhodopsin (FLEX-ArchT). Following a one-week recovery period, mice were handled daily for 5-7 days, then taken off of doxycycline for three days prior to context fear training. During context fear training, active neurons expressed ArchT and were tagged with H2B-GFP. Immediately after training, mice were placed back on doxycycline to prevent any non-specific tagging and were left undisturbed until testing day. 14 days later, mice were randomly assigned to a Laser-ON or a Laser-OFF group and tested in the same training context. Our preliminary data suggest that selective silencing of the tagged HPC ensemble (Laser-ON group) at 14 days does not impair memory retrieval, compared to the Laser-OFF group. Subsequent analyses will include a characterization of reactivation in the hippocampus and in corresponding cortical regions to determine which brain circuits are most critical in the successful retrieval of remote memories.

Disclosures: A. Hamidi: None. **Y. Ota:** None. **D. Mardini:** None. **S.L. Suarez:** None. **B.J. Wiltgen:** 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; RO1 NS088053 to B.J.W..

Poster

738. Memory Consolidation and Reconsolidation: Fear Conditioning Circuits

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Topic: H.01. Animal Cognition and Behavior

Support: Rebecca Cooper Medical Research Foundation

IHBI QUT

CSTS USU Bethesda

Title: Microcircuit level effects of MEK inhibitors on threat memory disruption

Authors: A. BATTLE[1], S. GILES[1], J. COYNER[2], R. J. URSANO[3], *L. R. JOHNSON[4]; [1]TRI IHBI QUT, Brisbane, Australia; [2]Program in Neurosci., USU Sch. of Med., Bethesda, MD; [3]Ctr. for the Study of Traumatic Stress, USU Sch. of Med., Bethesda, MD; [4]Translational Res. Institute, IHBI, QUT, Brisbane, Australia

Abstract: Memories formed as a result of threatening events can have debilitating effects on quality of life. Solutions to treat these memories are limited to behavioural therapies and non-specific pharmacotherapy. Neither behavioural therapies nor current pharmacotherapy have a lasting effect on memories and quality of life. Pavlovian threat (fear) conditioning results in changes to neural plasticity in the amygdala, specifically in the dorsal subdivision of the lateral amygdala (LAd), where plastic changes to the microcircuitry occur. Plastic changes include increases in the number of neurons expressing pMAPK/ERK activity and changes to the structure and shape of the neuron including changes to dendritic spines. Modifications to plasticity occur during both initial memory consolidation and subsequent reconsolidation of fear memories. MEK inhibitors, upstream inhibitors of pMAPK/ERK activity are known to block pMAPK/ERK activity when administered both intra brain and systemically and also block initial consolidation and reconsolidation of threat memories. However the precise effect of MEK inhibitors on different sub-regional microcircuitry is unknown. Our initial data suggests that the number of neurons in the LAd expressing pMAPK/ERK is significantly reduced (by up to 90%) following a systemic injection of the MEK inhibitor SL327. In conclusion these data provide initial microcircuit level analysis of the effects of MEK inhibitor on neural microcircuits underlying threat memories.

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Poster

738. Memory Consolidation and Reconsolidation: Fear Conditioning Circuits

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Title: Brain dynamics in fear memory: a small-animal Positron Emission Tomography study of its expression, labilization and reconsolidation.

Authors: *V. DE LA FUENTE[1], C. MEDINA[1], G. FALASCO[2], L. URRUTIA[2], S. VÁZQUEZ[2], M. E. PEDREIRA[1], A. ROMANO[1];
[1]Univ. of Buenos Aires & IFIBYNE-UBA-CONICET, Buenos Aires, Argentina; [2]Ctr. de Imágenes Moleculares, FLENI, Buenos Aires, Argentina

Abstract: It is now very well stated that consolidation is the process by which new information is encoded in neural circuits. However, once a memory is consolidated it does not remain stable indefinitely. Not only can it change with time but also with experience. In particular, when a reminder of the learning event is presented to an animal that has learnt something new, the memory and thus the neural circuits that encode that memory become labile and need a process of reconsolidation to be re-stabilized. This is crucial for the modification of existing memories as it enables changes in its strength and/or content. For the past decades, labilization/reconsolidation processes have been extensively studied from behavioral, cellular and molecular approaches but no whole-brain studies have emerged to elucidate neural circuits and brain areas subserving memory dynamics during these processes in small animals. In this work, we studied the male mouse brain from a functional perspective using small-animal Positron Emission Tomography (PET), and [18F]-deoxyglucose (FDG) as the radioactive compound, thus measuring differential glucose consumption. The main objective was to study which brain areas were involved in labilization/reconsolidation of fear memory using a contextual fear conditioning paradigm in mice. For that purpose we injected the mice with FDG intraperitoneally at different times pre/post re-exposure to the training context, then anesthetized the mice with isoflurane and acquired images through a PET scanner. We found differences in glucose consumption mainly in zones comprising the ectorhinal cortex, the temporal association cortex, hippocampus and amygdala in re-exposed animals compared to non-re-exposed animals. The differences in glucose consumption showed a marked temporal course, were context-specific, and were either hyper- or hypo-consumptions. Moreover, animals that only evoked but did not labilized the memory trace showed significant differences compared to mice that labilized and reconsolidated. Our work opens new insights in the study of the dynamics of activation of brain areas during memory labilization/reconsolidation by using a novel technique for the field, which in combination with others like immunofluorescence, DREADDS and electrophysiology helps to unravel the pending question about circuits involved in labilization/reconsolidation.

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Poster

738. Memory Consolidation and Reconsolidation: Fear Conditioning Circuits

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Support: SK project

JSPS KAKENHI (15H04258)

Title: Increased spine density in Arc-expressing neurons after contextual fear memory retrieval in the mouse hippocampus

Authors: K. MINATOHARA[1], M. AKIYOSHI[1], Y. TAKAHASHI[1], H. BITO[2], *H. OKUNO[1]; [1]SK project, Med. Innov Ctr., Kyoto Univ. Grad Schl of Med., Kyoto, Japan; [2]Dept. of Neurochemistry, The Univ. of Tokyo Grad. Sch. of Med., Tokyo, Japan

Abstract: The expression of immediate early genes (IEGs) such as *Arc* and c-*fos*, which is rapidly induced after learning in a subset of neurons in the brain, is required for long-term memory formation. These IEG-expressing neurons likely encode and retain information that is required for memory recall. When a memory is recalled, at least some of these neurons are reactivated, thus inducing IEGs that facilitate memory reconsolidation and updating. However, synaptic changes related to memory reconsolidation/updating in the neurons activated during memory retrieval remain unclear. To address this question, we analyzed morphological changes of dendritic spines in the activated neurons expressing *Arc* after contextual fear memory retrieval. We previously generated transgenic mice expressing a destabilized yellow fluorescent protein, Venus, with the C-terminal PEST sequence under the control of *Arc* promoter (Arc-pro-Venus-pest Tg mice) to specifically label neurons that express *Arc* in response to neuronal activation (Vousden et al., 2015). We trained these Tg mice in a contextual fear conditioning paradigm; the mice showed comparable ability with wild-type mice to retain fear memory assessed 24 hours after training. To evaluate the levels of *Arc* expression induced by memory retrieval in hippocampal CA1 pyramidal neurons, we quantified fluorescent intensities of the Venus reporter in the somata of the hippocampal neurons in brain sections fixed 2 hours after the retrieval test. This analysis revealed that the CA1 neurons displayed graded Venus intensities rather than an all-or-none profile, consistent with the immunofluorescence analysis for endogenous Arc expression. In order to visualize and analyze dendritic spine structures of these neurons, we sparsely labeled these neurons with a red fluorescent protein by adeno-associated viral infection, and classified them into two groups based on the Venus intensities in their cell bodies: high-Arc-expressing and low-Arc-expressing neuron groups. Comparing spine densities and spine morphology between these two groups revealed increased spine density and decreased spine head size in the high-Arc-expressing neurons ($P < 0.05$ for both; Mann-Whitney U test).

These results suggest that plastic changes in synaptic connections specific to the neurons activated during memory recall may play critical roles in updating and reorganizing previously acquired memory traces.

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Poster

739. Learning and Memory: Executive Functions

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Support: HHMI

Title: Rats accumulate and discount evidence in a changing environment

Authors: ***A. PIET**[1], C. BRODY[2], A. EL HADY[2];
[1]Neurosci., Princeton Univ., Princeton, NJ; [2]Neurosci., HHMI/Princeton Univ., Princeton, NJ

Abstract: How are choices made within constantly-changing noisy environments ? The gradual accumulation of noisy evidence is considered to be a fundamental component of decision making. Previous work has characterized the evidence accumulation process in a static environment, finding that rats and humans can accumulate evidence with a memory time constant longer than the trial duration (Brunton, Science 2013). However, complex decisions involve environments with statistics that change over time. In a changing environment, the optimal evidence accumulation strategy involves the additional task of discounting old evidence that may no longer inform the current state of the world (Glaze et al., eLife 2015; Veliz-Cuba et al., arXiv 2016; Barack et al., 2016). We trained rats on an auditory decision making task in a changing environment, to assess if and how rats can accumulate and discount evidence. Using high-throughput behavioral training and quantitative modeling, we find that rats can discount

evidence under such changing conditions. We are currently gathering data to determine the specific linear or nonlinear discounting strategy. Our study offers the first evidence that, within a dynamically changing environment, rats can perform a gradual evidence accumulation decision-making task. This opens up the opportunity to use rodents towards unraveling the complex interplay between accumulation and discounting of evidence in an environment where the statistical structure changes over time.

Disclosures: **A. Piet:** None. **C. Brody:** None. **A. El Hady:** None.

Poster

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Title: Flexible feature selection for decision-making in rats

Authors: ***M. PAGAN**[1], C. D. BRODY[1,2];
[1]Princeton Univ., Princeton, NJ; [2]Howard Hughes Med. Inst., Princeton, NJ

Abstract: A hallmark of higher cognition is our ability to flexibly select, based on context, the information relevant to guiding our decisions. Understanding the neural mechanisms underlying this fundamental cognitive process is a major unsolved question. To address this issue, we have devised a high-throughput, computer-automated procedure to efficiently train rats to perform a task requiring flexible, context-dependent selection and integration of sensory information (adapted from Mante et al., Nature, 2013). In our task, rats are presented with a 1.3s-long train of 5ms, randomly-timed auditory pulses. Each pulse is played either from a speaker to the animal's left or from a speaker to their right, and each pulse is either low-frequency (6.5 KHz) or high frequency (14 KHz). In blocks of "location" trials, rats are rewarded if they turn, at the end of the stimulus, towards the side that had played the greater total number of pulses, ignoring the frequency of the pulses. In blocks of "frequency" trials, rats are rewarded for orienting left if the total number of low frequency pulses was higher than the total number of high frequency pulses, and orienting right otherwise, ignoring the location of the pulses. For any given pulse train, correct performance thus requires selecting the relevant feature, depending on task context. Our preliminary behavioral data suggest that rats can learn to perform this behavior after

approximately 4 to 5 months of training. Because information is delivered in randomized, yet precisely-known pulses, statistical methods can be applied to precisely characterize the animals' behavior. Preliminary data suggest that the rate of accumulation of both context-relevant and context-irrelevant sensory information is approximately constant throughout the stimulus presentation. In summary, our results demonstrate that rats are a viable model to study context-dependent feature selection for decision-making, and open the door to the use of the rich experimental tools available in rodents to dissect the neural mechanisms underlying flexible feature selection.

Disclosures: M. Pagan: None. **C.D. Brody:** None.

Poster

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Topic: H.01. Animal Cognition and Behavior

Title: Trial history vs. sensory memory - a causal study of the contribution of rat Posterior Parietal Cortex (PPC) to history-dependent effects in working memory

Authors: *A. AKRAMI[1,2], C. D. KOPEC[1], C. D. BRODY[1,2];
[1]Princeton Neurosci. Inst. (PNI), Princeton, NJ; [2]Howard Hughes Med. Inst., Princeton, NJ

Abstract: Behavior in various tasks cannot be seen as an isolated phenomenon out of the context, that is, the brain is constantly engaged in storing new memories and executing actions while integrating incoming sensory input with past memories and subject's internal model of the task. Working memory (WM), the ability to store and manipulate information for short periods of time, is an example where contextual information, given specifics of the past history, becomes an integral part of perception and memory. A particular type of WM task, called Parametric Working Memory (PWM), is delayed comparison, the sequential comparison of two graded stimuli separated by a delay period of a few seconds, which forces the subject to maintain an analog value in memory. We have developed an auditory delayed comparison task in rats, adapted from a tactile task (Fassihi and Akrami et. al 2014, Akrami et al 2015). On average, rats show a remarkable ability to hold information about the first stimulus in their memory for up to 10s. A salient feature of PWM is that it is adaptive to various factors in the task, e.g the saliency of the stimuli, the delay duration, and interestingly, task history. Using a regression model to quantify the animals' behavior, we show how performance on each trial depends on the reward history (Busse et al 2011), as well as the stimulus history. These biases are similarly observed in human subjects performing a similar PWM task. For the first time we carry out local optogenetic

inactivations of Posterior Parietal Cortex (PPC) in rats which is assumed to be involved in working memory. We found that, surprisingly, some PPC inactivations improve performance. Quantitative analyses suggest that this may be due to a reduction in the biases caused by the history of recent trial rewards and punishments, as well as sensory stimuli. Furthermore, electrophysiological recordings from PPC does not show its involvement during the delay period but rather show neurons carry significant amount of information about the subject's recent experience of sensory stimuli as well as their recent choices and rewards. These results suggest a crucial history-dependent contribution of PPC to working memory behaviors and specifically to longer scale memory of past sensory inputs that span over several trials.

Akrami A, El Hady A, Kopec CD, Brody C (2015) Time dependent involvement of Posterior Parietal and Prefrontal cortex in a rat auditory parametric working memory task, SfN2015.
Fassihi* A, Akrami* A, Esmaeili V, Diamond ME (2014) Tactile perception and working memory in rats and humans. *PNAS*.
Busse, Laura, et al (2011) "The detection of visual contrast in the behaving mouse." The Journal of neuroscience

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Poster

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Support: NIH U01 NS090541

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Title: Systematic optogenetic mapping of cortical regions required for sensory-evidence accumulation in the mouse

Authors: ***L. PINTO**[1], S. KOAY[1], S. Y. THIBERGE[1,2], D. W. TANK[1,2], C. D. BRODY[1,3]; [1]Princeton Neurosci. Inst., [2]Bezos Ctr. for Neural Dynamics, [3]Howard Hughes Med. Inst., Princeton Univ., Princeton, NJ

Abstract: The accumulation of sensory evidence is a crucial aspect of perceptual decision-making, and it involves complex neural computations requiring sensory processing, weighing of sensory evidence for or against a decision, as well as short-term memory of accrued evidence. Given this complexity, it is likely that many cortical areas are causally involved in evidence

accumulation. However, little is known about which areas are necessary for this crucial cognitive function. To tackle this question, we implanted VGAT-ChR2-EYFP mice with a transparent skull preparation, allowing optical access to the entire dorsal surface of the cortex, and inactivation of discrete cortical patches by activating ChR2-expressing GABAergic neurons (Guo et al., 2014, Neuron). Head-fixed mice were trained on a novel virtual navigation-based visual accumulation task (Koay et al., SFN Abstracts 2015; see also Koay et al., SFN 2016), wherein they navigate a T-maze to retrieve water rewards from one of the two arms. While the animals run up the central stem of the virtual T salient visual stimuli (vertical columns, or towers) appear on either side, distributed as a spatial Poisson process of different rates (cue region, 200 cm). After a memory region without any stimuli (100 cm), the animal then makes a right or left to turn to indicate which side had the highest total number of towers. The visual stimuli thus need to be incrementally accumulated towards a decision. Mice achieved high overall performance (~ 80% correct), were sensitive to the difference in right and left towers, and incrementally accrued evidence, as revealed by psychometric functions and a psychophysical reverse correlation analysis (Brunton et al., 2013, Science). We then used a blue laser coupled to a 2D scanning galvanometer system to comprehensively and systematically inactivate discrete cortical patches while mice performed the task. Whole-trial inactivation of several visual cortical regions and association cortex resulted in decreases in performance, observed in VGAT-ChR2-EYFP mice but not in control mice. We are currently carrying out finer temporal inactivation, e.g. only during the cue or memory periods, to allow us to tease apart the individual contributions of these areas to specific computations in the task. Identifying these regions will be crucial to guide future cellular-resolution studies to understand the neural circuit computations underlying evidence accumulation for decision-making.

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NARSAD Young Investigator

Title: Recurrent network models of evidence accumulation.

Authors: *K. RAJAN, B. B. SCOTT, C. M. CONSTANTINOPLE, D. W. TANK;
Princeton Univ., Princeton, NJ

Abstract: Behavioral experiments have raised a number of quantitative, testable hypotheses about how the brain encodes accumulated evidence in favor of a decision, in both rodents [Brunton, Botvinick & Brody, 2013; Scott et al, 2015], and primate studies [Gold & Shadlen, 2007]. Experimental advances now support recordings and imaging in the frontal orienting field, secondary visual cortex and posterior parietal cortex during accumulation of evidence tasks, [Scott et al, in prep; Hanks et al, 2015]. Here, we combine analysis of the resulting population data with neural network modeling of both the behavioral process and the neuronal dynamics to identify mechanisms in cortical circuits that provide the type of working memory involved in evidence accumulation [Pereira & Wang, 2014; Wang, 2012]. In most models, memories are stored over short periods of time by persistent neural activity, particularly through fixed points [Hopfield & Tank, 1985; Hopfield, 2015; Amit & Brunel, 1995; Hansel & Mato, 2001]. Recently, an alternative mechanism has been suggested involving richer patterns of population activity - neural sequences [Rajan, Harvey & Tank, 2016]. We have studied the role of neural sequences in mediating memory for evidence accumulation and discrimination tasks. The main hypothesis is that diverse and transient sensory responses distributed across a neural population encode accumulated evidence in working memory during these tasks.
To test this hypothesis, we trained recurrent networks of model neurons to perform evidence accumulation tasks based on comparison of stimuli of two different magnitudes. The trained networks produce sequential activity to support short-term memory and contain both partially structured connectivity for stimulus integration and evidence accumulation, combined with random connectivity to stabilize other functions within a reservoir computing framework. We evaluate these networks based on the degree to which they match the observed trial-by-trial variability and scaling of the estimation errors in modeling the behavioral data [Nieder & Miller, 2003; Brunton, Botvinick & Brody, 2013; Scott et al, 2015]. In addition, networks built in this manner capture the behavioral performance of the rats, matching measured psychometric functions. By incorporating both reward-incentivized exploration in the training process and inattention/distraction inputs, the model can also be used to investigate the neural mechanisms underlying “lapse rates” observed experimentally during these and similar tasks.

Disclosures: K. Rajan: None. **B.B. Scott:** None. **C.M. Constantinople:** None. **D.W. Tank:** None.

Poster

739. Learning and Memory: Executive Functions

Location: Halls B-H

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Program#/Poster#: 739.06/HHH33

Topic: H.01. Animal Cognition and Behavior

Support: NIH R01-NS045193

NIH U01 NS090541

Title: A head-fixed somatosensory task in mice for the study of neural mechanisms of evidence accumulation

Authors: *B. DEVERETT[1,2,3,4], S. KOAY[1,2], S. S.-H. WANG[1,2,3];
[2]Princeton Neurosci. Inst., [3]Dept. of Mol. Biol., [1]Princeton Univ., Princeton, NJ; [4]Rutgers-Robert Wood Johnson Med. School-Princeton Univ. MD/PhD Program, Rutgers Univ., New Brunswick, NJ

Abstract: Working memory is a core cognitive process that supports perception, decision making, and problem solving. In animal models, neural and behavioral dynamics of working memory can be probed through the gradual presentation of sensory evidence over time. For example, rodents can be trained to accumulate evidence in the form of pulsatile visual or auditory stimuli presented on their left and right sides (Brunton et al. 2013, Science 340.6128:95-8; Scott et al. 2015, eLife 4.e11308, Koay et al., Soc. Neurosci. Abstr. 2015, 442.15/BB47). In these tasks, rodents indicate the side with the greater amount of evidence by orienting or navigating, both of which involve a specific motor action.
We established a head-fixed mouse paradigm for working memory that deviates from these tasks in two ways: the modality of sensory stimuli and the mode of decision readout. In this new task, head-fixed mice are presented with sensory evidence in the form of brief puffs of air to the whisker pad regions of the face, with onset times distributed randomly according to a Poisson process with different rates for left- and right-sided stimuli. Mice are taught to suppress licking while evidence is presented during a period that varies in duration from trial to trial. After an additional delay period, a visual “go” cue is presented, and mice report their choice by licking one of two liquid reward delivery tubes positioned to their left and right. Mice learned to perform this task at levels comparable to head-orienting and virtual reality-based paradigms. After approximately twenty one-hour sessions, mice successfully performed trials ranging from 10-16 seconds in duration, with stimulus rate differences ranging from 9:1 to 3:2. Psychometric, chronometric, and reverse correlation analyses suggest that mice integrated information across the whole cue presentation period. In comparison to a virtual reality-based visual evidence accumulation task, mice exhibited lower lapse rates and higher consistency. This task may help disambiguate higher-level cognitive features from movement-specific and sensory modality-

specific effects. In particular, we are using this evidence accumulation task to explore contributions of cerebellar regions that have strong connectivity with nonmotor neocortical regions involved in working memory (Wang, Kloth, & Badura 2014, Neuron 83:518, Strick et al. 2009, Annu. Rev. Neurosci. 32.1:413-34).

Disclosures: B. Deverett: None. **S. Koay:** None. **S.S. Wang:** None.

Poster

739. Learning and Memory: Executive Functions

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Topic: H.01. Animal Cognition and Behavior

Support: NIH grant U01 NS090541

Simons Foundation SCGB

Title: Neural dynamics in a mouse navigation and accumulation of visual evidence task

Authors: *S. KOAY[1], B. ENGELHARD[1], L. PINTO[1], B. DEVERETT[1,2,3], S. Y. THIBERGE[1,4], C. D. BRODY[1,5], D. W. TANK[1,4,2];
[1]Princeton Neurosci. Inst., Princeton, NJ; [2]Dept. of Mol. Biol., Princeton, NJ; [3]Rutgers-Robert Wood Johnson Med. School-Princeton Univ. MD/PhD Program, Piscataway, NJ; [4]Bezos Ctr. for Neural Circuit Dynamics, Princeton, NJ; [5]Howard Hughes Med. Inst., Princeton, NJ

Abstract: Decision-making tasks involving accumulation of sensory evidence provide a flexible framework in which to study neural mechanisms of working memory. Extending work in human and non-human primates (Gold et al., Annu Rev of Neuroscience 2007) and rats (Brunton et al., Science 2013; Scott et al., eLife 2015), we demonstrated that mice can perform a combined virtual reality navigation and accumulation of visual evidence decision-making task (Koay et al., SFN 2015). Transgenic mice expressing the genetically encoded calcium sensor GCaMP6f in excitatory neurons were trained in a head-fixed virtual reality system (Harvey et al., Nature 2009) to navigate in a T-shaped maze. As mice ran down the main corridor, visually salient "towers" appeared along the left and right walls of the cue region. Tower positions were randomly distributed per trial as spatial Poisson processes with a different mean count for each side. Mice then traversed a memory region where no towers appeared. Upon reaching the T-intersection they had to run down the left or right arm (turn region), and were given a liquid reward for selecting the side that had more towers. Mice achieved performances of 70%-80% correct trials, with trial durations of 10-20s. Each mouse was implanted with a 5mm diameter

cranial imaging window centered over the anterior-medial border of primary visual cortex, permitting two-photon cellular resolution imaging of calcium dynamics of neurons in layers 2/3 and 5 across a wide region of cortex. Neural activity underlying calcium dynamics was estimated using a demixing and deconvolution algorithm (Pnevmatikakis et al., Neuron 2015). Recording locations (AM, retrosplenial cortex, etc.) were defined relative to primary and secondary visual areas whose borders were estimated using wide-field functional mapping (Garrett et al., J. Neuroscience 2014). In most regions we observed neurons with task-modulated activity; cells typically had Ca^{2+} transients for only short time intervals (hundreds of ms to seconds) that within a trial were staggered in time across the population (Harvey et al., Nature 2012). Regional differences were seen in the frequency of cells active in the cue, memory, and turn regions. Of particular interest to evidence accumulation models, some neurons exhibited transient responses to the presentation of individual towers, predominantly for towers on a preferred side. Response amplitudes were highly variable and often had different overall modulations which depended on position in the cue region. These experiments yield a rich dataset for examining neural coding and dynamics underlying evidence accumulation and decision-making.

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Poster

739. Learning and Memory: Executive Functions

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Topic: H.01. Animal Cognition and Behavior

Support: NIH grant R21NS082956

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Howard Hughes Medical Institute

Title: Heterogeneous dynamics in frontoparietal cortex encode sensory memories during accumulation of evidence tasks

Authors: ***B. B. SCOTT**[1,2,3], C. M. CONSTANTINOPLE[1,2,3], C. D. BRODY[1,2,3,4], D. W. TANK[1,2,3];
[1]Mol. Biol., [2]Princeton Neurosci. Inst., [3]Bezos Ctr. for Neural Circuit Dynamics, [4]Howard Hughes Med. Inst., Princeton Univ., Princeton, NJ

Abstract: Behavioral studies suggest that animals accumulate sensory evidence over time to make perceptual decisions. In mammals this accumulation process is thought to involve the activity of neurons in frontal and parietal neocortex, however the precise circuit mechanisms of evidence accumulation are not known. Using two-photon imaging in voluntarily head restrained rats (Scott et al., Neuron, 2013), we examined calcium dynamics of GCaMP6f-expressing layer 2/3 cortical neurons during a pulse-based accumulation of evidence task, which we recently developed (Scott, Constantinople et al., eLife, 2015). In this task rats viewed two simultaneous streams of light pulses, presented from a left and a right LED. After the end of the streams, rats were rewarded for orienting to the side that had the greater number of pulses. A linear encoding model was used to characterize the responses of neurons to task events including individual quanta (flashes) of evidence, and to distinguish sensory responses from premotor activity. Consistent with previous studies, a population of neurons distributed across frontal and parietal cortex exhibited strong responses that reflected both the timing and the magnitude of the accumulated sensory evidence. However, contrary to leading models of evidence accumulation, these neurons displayed extensive heterogeneity in the dynamics of responses to pulses. Responses of individual neurons were transient, typically shorter than the duration of the trial, with different neurons active at different times following a pulse. Inspired by network models of working memory in which transient and heterogeneous responses combine linearly to produce persistent activity (Goldman, Neuron, 2009), we found that the temporal diversity of responses was sufficiently rich to form a basis set for a wide range of exponential decay functions, including those with long time constants. These results suggest that diverse, time varying sensory responses distributed across frontoparietal cortex may support the parametric visual working memory necessary for evidence accumulation on behavioral timescales.

Disclosures: **B.B. Scott:** None. **C.M. Constantinople:** None. **C.D. Brody:** None. **D.W. Tank:** None.

Poster

739. Learning and Memory: Executive Functions

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Program#/Poster#: 739.09/HHH36

Topic: H.01. Animal Cognition and Behavior

Support: R01MH065658

Title: Stable contingencies drive orbitofrontal predictions

Authors: *J. S. RICEBERG[1], M. SHAPIRO[2];
[1]Neurosci., Mount Sinai Sch. of Med., New York, NY; [2]Icahn Sch. of Med. of Mount Sinai, New York, NY

Abstract: Memory can inform goal-directed behavior by linking current opportunities to past outcomes. The orbitofrontal cortex (OFC) may guide value-based responses by integrating the history of stimulus-reward associations and computing expected outcomes: representations of predicted hedonic value and quality. We compared OFC coding in identical tasks controlled by contingencies that varied reward history. Under stable contingencies the OFC signaled integrated reward history with anticorrelated population codes that distinguished goals and predicted task performance. The firing rates of single neurons distinguished identical behaviors guided by different outcomes in predictive, retrospective, and goal location coding. When contingencies were unstable, however, the OFC signaled different outcomes with uncorrelated population codes that did not predict performance, and though retrospective and goal coding were maintained, predictive coding declined. The OFC distinguishes recent actions and actual outcomes independent of reward stability, but predicts expected outcomes only when stable contingencies promote the integration of reward history.

Disclosures: J.S. Riceberg: None. **M. Shapiro:** None.

Poster

739. Learning and Memory: Executive Functions

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Program#/Poster#: 739.10/HHH37

Topic: H.01. Animal Cognition and Behavior

Support: R01MH102394

Title: Distinct contributions of hippocampal and prefrontal afferents to nucleus reuniens during spatial working memory

Authors: B. A. EMANUEL, H. L. HALLOCK, E. C. MYHRE, *A. L. GRIFFIN;
Univ. Delaware, Newark, DE

Abstract: Spatial working memory, the ability to acquire, maintain, and use spatially relevant information over a temporal gap, has been demonstrated to depend on functional interactions between the hippocampus and medial prefrontal cortex (mPFC). Recently, our lab has demonstrated that the nucleus reuniens (Re), part of the ventral midline thalamus that is reciprocally connected to both hippocampus and mPFC, plays a crucial role in coordinating

interactions within the hippocampal-mPFC circuit during spatial working memory (SWM). However, the specific circuitry that governs this coordination has not yet been explored. Therefore, we investigated the functional involvement of Re and its afferents in SWM using a projection-targeting optogenetic silencing approach. We infused an adeno-associated virus vector engineered to express fluorescently labelled archaerhodopsin (pAAV5-CAG-ArchT-GFP) into the dorsal subiculum, mPFC, or Re in separate groups of rats. After virus injection, all rats were implanted with an optical fiber targeting the Re. Rats were then allowed to recover for at least 6 weeks to allow for opsin expression. We then trained rats to perform a delayed nonmatch to position (DNMP) task in a T-maze. The advantage of using a DNMP task is that its design effectively teases apart the encoding and retrieval portions of SWM into two separate traversals of the maze: sample (encoding) and choice (retrieval) phases, separated by a delay period over which information must be maintained. After rats reached criterion on the DNMP task, data collection began. Optical stimulation was driven via a compact LED module emitting 550-nm light through a patch cable. Choice accuracy was recorded from four distinct experimental conditions given in pseudorandom order across days that varied according to which task phase the rat was performing when the LED was turned on: the sample phase, the choice phase, the delay period and the entire trial. The Re group showed impaired choice accuracy on all four conditions. Terminal suppression of the dorsal subicular input to Re caused a selective deficit on the sample-only light condition, suggesting that dorsal subiculum-Re projections are critical for the encoding of task-relevant spatial cues. Conversely, terminal suppression of mPFC input to Re caused a selective deficit on the delay-only light condition, suggesting that medial prefrontal-Re projections support the maintenance of these spatial representations. These findings implicate Re in all aspects of spatial working memory, and indicate pathway-specific roles for Re afferents during the encoding and maintenance of spatial information necessary for successful SWM.

Disclosures: **B.A. Emanuel:** None. **H.L. Hallock:** None. **E.C. Myhre:** None. **A.L. Griffin:** None.

Poster

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Topic: H.01. Animal Cognition and Behavior

Support: R01MH102394

Title: Investigating the role of the nucleus reuniens in spatial working memory

Authors: *A. C. GARCIA, A. L. GRIFFIN;
Univ. of Delaware, Newark, DE

Abstract: Spatial working memory (SWM) has been shown to rely on the functional integrity and connectivity of the hippocampus and medial prefrontal cortex (mPFC). However, it remains to be determined how task-relevant information is organized within this circuit to guide SWM behavior. In the rat, direct connections between the hippocampus and mPFC are restricted to a monosynaptic projection extending from the ventral portion of hippocampus to mPFC. However, the nucleus reuniens (RE) of ventral-midline thalamus has been shown to share afferents and efferents with both dorsal and ventral hippocampus and mPFC. Recent work from our lab using *in vivo* recordings of RE in the awake, behaving rat has shown that disruption of RE activity not only impairs SWM performance, but concomitantly disrupts hippocampal-mPFC synchrony and the direction of information flow within the hippocampal-mPFC circuit. Thus, RE could play a role in coordinating hippocampal-mPFC interactions during SWM. Consistent with this notion, a recent study demonstrated that suppression of RE activity decreased trajectory-dependent firing in hippocampal CA1 during a continuous alternation (CA) task (Ito et al., 2015). However, compared to other SWM tasks, both the SWM demand of the CA task and its ability to dissociate between the processes of encoding and retrieval are relatively low. Moreover, experimental evidence shows that the CA task does not require the functional integrity of the hippocampus for successful performance. Therefore, we set out to investigate the role of RE in SWM using a hippocampus-dependent task with a high SWM demand that allows dissociation between the processes of encoding and retrieval: the delayed non-match to position (DNMP) task in a T-maze. This task requires cue-based encoding on the sample run, maintenance of the information during a 20-second delay period, and memory-dependent retrieval on the choice run. In line with previous reports, a subset of RE units showed trajectory-dependent firing, while a separate subset showed differential firing between sample and choice runs, reminiscent of CA1 single units recorded during a DNMP task (Griffin, Eichenbaum, & Hasselmo, 2007). These results suggest that distinct subsets of RE units differentially contribute to encoding and retrieval processes. Therefore, RE may function as a hub for integrating information from multiple brain regions and dynamically directing task-relevant information within the hippocampal-mPFC circuit to support SWM-guided decision making.

Disclosures: A.C. Garcia: None. **A.L. Griffin:** None.

Poster

739. Learning and Memory: Executive Functions

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Topic: H.01. Animal Cognition and Behavior

Support: HHMI

NIH

Title: Neural ensemble dynamics of the medial prefrontal cortex underlying shifts in cognitive strategy

Authors: *F. WANG, T. H. KIM, H. INAN, A. WILLIAMS, S. GANGULI, M. J. SCHNITZER;
Stanford Univ., Stanford, CA

Abstract: During shifts in cognitive strategy, the medial prefrontal cortex (mPFC) is thought to exert a crucial role by coordinating the functions of multiple brain systems. Inactivation of the mPFC impairs the ability to shift between different cognitive strategies[1-3], and electrophysiological recordings in behaving rodents have revealed evidence of mPFC neural activity that is temporally linked to strategy shifts[4,5]. To examine the neural ensemble dynamics underlying strategy shifts, we used a miniature fluorescence microscope to image the calcium dynamics of hundreds of individual mPFC neurons in the prelimbic cortex (PrL) of mice performing a strategy-switching navigation task.
In this task, mice switched between a hippocampus-dependent, allocentric (spatial) navigation strategy and a striatum-dependent, egocentric strategy (or vice versa) to earn a water reward. We identified distinct subsets of PrL neurons with either contemporaneous or anticipatory coding for various task parameters and phases, including the mouse's spatial location and the anticipated receipt of reward. We also found neural ensemble activity patterns in PrL related to the two different strategies, and we are presently studying how these patterns and their dynamics might underlie the representations and shifts between different strategies. Overall, our work reveals the large-scale dynamics of frontal cortical neural ensembles in mice performing our specific cognitive task, and paves the way to future studies of the neural ensemble codes underlying other modes of cognitive control.
1. Birrell & Brown (2000) Medial Frontal Cortex Mediates Perceptual Attentional Set Shifting in the Rat. J Neurosci. 20(11):4320-43242. Floresco et al. (2008) Inactivation of the medial prefrontal cortex of the rat impairs strategy. Behavioural Brain Research. 190:85-963. Ragozzino et al. (1999) Involvement of the Prelimbic-Infralimbic Areas of the Rodent Prefrontal Cortex in Behavioral Flexibility for Place and Response Learning set-shifting, but not reversal learning, using a novel, automated procedure. J Neurosci. 19(11):4585-45944. Rich & Shapiro (2009) Rat prefrontal cortical neurons selectively code strategy switches. J Neurosci. 29(22):7208-7219.25. Durstewitz et al. (2010) Abrupt Transitions between Prefrontal Neural Ensemble States Accompany Behavioral Transitions during Rule Learning. Neuron. 66, 438-448.

Disclosures: F. Wang: None. **T.H. Kim:** None. **H. Inan:** None. **A. Williams:** None. **S. Ganguli:** None. **M.J. Schnitzer:** Other; Inscopix Inc..

Poster

739. Learning and Memory: Executive Functions

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Topic: H.01. Animal Cognition and Behavior

Support: Medical Research Council Senior Non-Clinical Fellowship (to M.D.H.)

Title: Outcome selective recall of neural ensembles in prefrontal cortex of rule-learning rats

Authors: *S. MAGGI[1], A. PEYRACHE[2,3], M. D. HUMPHRIES[1];
[1]Fac. of Life Sci., Univ. of Manchester, Manchester, United Kingdom; [2]NYU Med. Ctr., New York, NY; [3]McGill Univ., Montreal, QC, Canada

Abstract: The medial prefrontal cortex (mPFC) contributes to diverse aspects of memory-driven cognition, including working memory during tasks, and replay of task-related activity during sleep. Unclear is how mPFC population dynamics underlie working memory contributions to learning. One possibility is that mPFC populations support learning using cell assemblies that appear in order to sustain relevant items in working memory.
To address these issues, we analysed population activity from the mPFC of rats learning rules in a Y-maze. Spike-train data (tetrode recordings) and behavioural data were from the study of Peyrache et al (2009; Nat Neurosci, 12, 916-926). Rats were required to learn one of three rules on the Y-maze: go left; go right; or go to the lit arm. Reward was obtained by reaching the end of the goal arm corresponding to the current rule. After reward delivery or absence, rats made a self-paced return to the starting position during the inter-trial interval (ITI), which was examined for signatures of learning-specific working memory. We analysed data from 46 sessions, in 10 of which the rats met the learning criterion mid-session.
To test for signatures of task-specific working memory, we quantified the functional network of pairwise correlations between simultaneously recorded neurons in the ITI. In every session, we found the functional network was comprised of statistically robust neural ensembles. Within each session, ensembles were present in every ITI and formed a remarkably stable proportion of the population across all ITIs. Having detected potential cell assemblies, comparing learning and non-learning sessions allowed us to test for their role in working memory for learning. Remarkably, we found that the same ensembles were recalled after each correct trial, but not error trials. This outcome-selective recall only occurred during learning sessions. Moreover, within each learning session, the appearance of outcome selective ensembles preceded stable behavioural performance. Our results show that mPFC populations form true cell assemblies during learning, which retrospectively code for rewarding outcomes. These assemblies could provide the dynamical basis for maintaining a specific task-related item in working memory when triggered by reward.

Disclosures: **S. Maggi:** None. **A. Peyrache:** None. **M.D. Humphries:** None.

Poster

739. Learning and Memory: Executive Functions

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Topic: H.01. Animal Cognition and Behavior

Title: Evaluating the role of GABA interneurons in the medial prefrontal cortex during working memory in mice

Authors: ***R. NGUYEN**[1], J. C. KIM[2];
[1]Psychology, [2]Univ. of Toronto, Toronto, ON, Canada

Abstract: Working memory involves coordinated timing and synchrony of network activity in the medial prefrontal cortex (mPFC) by GABA interneurons. GABA interneurons are a diverse population with distinct cell types differentially contributing to cognition and behaviour. Two major non-overlapping GABA interneuron populations in the mPFC are the parvalbumin (PV)- and cholecystokinin (CCK)- GABA interneurons. We examined the requirement of PV and CCK-GABA interneurons in working memory by optogenetically silencing their activity in the mPFC of mice performing an olfactory delayed-nonmatch-to-sample test. The inhibitory opsin ArchT was expressed in CCK-GABA interneurons using dual recombinase intersectional genetic labeling, and in PV interneurons using AAV-mediated gene delivery. PV and CCK-GABA interneurons were inhibited either during the sample, delay, or response phase of the task by light illumination timed to these events. CCK-GABA interneuron inhibition selectively during the response phase impaired working memory performance. These results support the function of CCK-GABA interneurons in working memory retrieval or expression.

Disclosures: **R. Nguyen:** None. **J.C. Kim:** None.

Poster

739. Learning and Memory: Executive Functions

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Topic: H.01. Animal Cognition and Behavior

Support: NSERC Discovery Grant 355868-20

Title: Performance of the odour span task is not disrupted by inactivations of the posterior parietal cortex in rats

Authors: *G. A. SCOTT, N. K. ZABDER, Q. GREBA, J. G. HOWLAND;
Dept. of Physiol., Univ. of Saskatchewan, Saskatoon, SK, Canada

Abstract: Working memory, the ability to temporarily store information for use and manipulation, is thought to depend on a distributed set of brain areas. These include higher cortical areas involved in executive function and attention, such as the prefrontal and parietal cortices, which maintain attention on the items stored in working memory. However, relatively little research has been conducted in rodents to elucidate the exact roles each of these areas plays in working memory, particularly in relation to the construct of working memory capacity. Previous work in our lab has demonstrated that performance of the odour span task (OST), a behavioural task that tests the ability of rats to remember an increasingly large, trial unique set of odours in order to obtain a food reward, in rats is disrupted by the temporary inactivation of the medial prefrontal cortex and striatum. However, the effects of deactivating the parietal cortex on the OST has not been studied. Therefore, the present experiment assessed the effects of inactivating the posterior parietal cortex on performance of the OST. Rats (n = 12) were food restricted and, after being shaped to perform the basic rules of the task, underwent training in the OST over a period of 1.5-3 weeks. Four guide cannulae were then surgically implanted aimed at the posterior parietal cortex (AP -3.8 mm; ML ±2.2 mm and 3.4 mm; DV -0.2 mm from brain surface). After recovery and retraining, rats were tested after intra-parietal infusions of a mixture of muscimol and baclofen (GABA-A and GABA-B agonists, respectively) or saline (control). The infusions took place over two days using a crossover design in which each rat received muscimol/baclofen and saline in a counterbalanced order. Interestingly, infusions of muscimol/baclofen did not disrupt working memory performance, assessed by the mean number of odours each rat could remember before making an error on each day of training. A subset of rats (n = 4) was also tested in crossmodal object recognition (CMOR; a test that depends on the parietal cortex) as a positive control, and exhibited a memory impairment after muscimol/baclofen, but not saline, infusions. These results suggest that performance of the odour span task does not depend on the parietal cortex. The result is surprising given the past research

demonstrating the importance of the parietal cortex for attentional processes and working memory in other tasks. Current work to expand the sample size in CMOR is ongoing.

Disclosures: **G.A. Scott:** None. **N.K. Zabder:** None. **Q. Greba:** None. **J.G. Howland:** None.

Poster

739. Learning and Memory: Executive Functions

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Program#/Poster#: 739.16/III3

Topic: H.01. Animal Cognition and Behavior

Support: SHRF 3161

NSERC 355868-20

Title: The role of the medial prefrontal cortex in working memory capacity

Authors: ***L. AN**[1], N. SUN[1], J. CATTON[1], A. TSENGER[1], Q. GREBA[1], C. LAPISH[2], J. HOWLAND[1];
[1]Univ. of Saskatchewan, Saskatoon, SK, Canada; [2]Indiana Univ., Indianapolis, IN

Abstract: Working memory is a psychological construct encompassing executive functions responsible for the manipulation and maintenance of information over short intervals. The amount of information that can be held is referred to as working memory capacity. In rodents, the medial prefrontal cortex (mPFC) plays a key role in organizing and processing cognitive functions, including working memory capacity. To better understand how mPFC neurons contribute to working memory capacity, 13 male Long-Evans rats were trained on the odor span task (OST), a non-spatial task that assesses the capacity of working memory. Following implantation of electrode arrays, ensemble extracellular recordings of single units were obtained from the mPFC of freely moving rats during the OST. Neurons (n=394) with a mean baseline firing rate of 4.7±1.1 Hz were collected. Approximately 80% of the units were classified as regular-spiking (RS) cells and about 8% of them were fast-spiking (FS) interneurons. We explored the roles of mPFC neurons during sequential events of a given trial (trial start, odor approach, and reward) and the delay period under different spans (low, 2-6 odors; medium, 8-11 odors; high, 13-17 odors). Firing rate of RS cells increased during trial start, before novel and familiar odor approach, and after obtaining reward. Interestingly, firing rates significantly changed when correct, but not incorrect, choices were made. About 60% of event-related units significantly changed their activity to more than one event, and this proportion tended to increase with increasing span. No obvious event-related changes were found in the firing pattern among

low, medium, and high spans. During the delay, RS neurons were detected that increased activity during the start of the delay, end of the delay, or throughout the delay period. Delay cells that increased firing rate before trial start changed their firing pattern in the late period of the high span trials with greater activity 1 s before trial start. These preliminary data provide insight into the neural dynamics of working memory, specifically during correct choices.

Disclosures: **L. An:** A. Employment/Salary (full or part-time): Dept. of Physiology, University of Saskatchewan, Saskatoon, SK, Canada. **N. Sun:** None. **J. Catton:** None. **A. Tsenger:** None. **Q. Greba:** None. **C. Lapish:** A. Employment/Salary (full or part-time): Department of Psychology, Stark Neuroscience Institute, Institute for Mathematical Modeling and Computational Sciences, Indiana University—Purdue University Indianapolis, Indianapolis, Indiana, US. **J. Howland:** A. Employment/Salary (full or part-time): Dept. of Physiology, University of Saskatchewan, Saskatoon, SK, Canada.

Poster

740. Transcriptional and Epigenetic Mechanisms of Learning and Memory

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Topic: H.01. Animal Cognition and Behavior

Title: Holeboard discrimination learning in S100 calcium-binding protein B knockout mice

Authors: K. HAGEBOUTROS[1], H. WU[2], V. VENKATARAMAN[2], *B. D. FISCHER[1]; [1]Cooper Med. Sch. of Rowan Univ., Camden, NJ; [2]Rowan Univ. Sch. of Osteo. Med., Stratford, NJ

Abstract: The holeboard learning test is a well-established measure of spatial learning, which has been used in both genetic and pharmacological mouse models. The apparatus is an open field chamber with 16 holes in the floorboard. The task requires mice to discriminate between baited vs. non-baited holes over successive trials. The holeboard test assesses spatial working memory and reference memory. S100β is a calcium-binding astroglial protein whose gene is found on chromosome 21. The overexpression of S100β is implicated in Alzheimer's disease and Down syndrome. The present study used the holeboard test to assess learning and memory expression in mice deficient in S100β (S100β KO mice). Our working hypothesis is that lacking S100β serves as a protective mechanism in regards to memory. After a habituation phase, food-deprived mice were trained for three consecutive days. Each day consisted of 6 consecutive 3-minute trials. A delay recall test was conducted three weeks later with the same baited holes. Working memory errors (entering a hole that was already visited) and reference memory errors (visiting a hole that is not baited) were recorded. The S100β KO mice readily acquired the task and both

working- and reference-memory errors were reduced by day two. There was no significant difference between 3 month old and 9 month old mice in terms of number of reference and working memory errors made across trials. Overall, the low average number of memory errors made and lack of statistical significance between 3 month old and 9 month old mice in the present study raise the possibility that lacking S100β can serve as a protective mechanism against memory decline.

Disclosures: K. Hageboutros: None. **H. Wu:** None. **V. Venkataraman:** None. **B.D. Fischer:** None.

Poster

740. Transcriptional and Epigenetic Mechanisms of Learning and Memory

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 740.02/III5

Topic: H.01. Animal Cognition and Behavior

Support: JSPS KAKENHI (25340066)

Title: Role of toll-like receptor 4 in diesel exhaust origin secondary organic aerosol-induced impaired spatial learning ability in neonatal mice

Authors: *T. WIN SHWE, Y. FUJITANI, H. WATANABE, S. HIRANO;
Natl. Inst. Envir, Tsukuba, Japan

Abstract: *Background:* Recently, we have reported that diesel exhaust origin secondary organic aerosol (DE-SOA) exposure during developmental period may affect the olfactory-based spatial learning behavior in preweaning mice. Toll-like receptor (TLR)-4, a pathogen-associated molecular pattern receptor plays a role in inflammatory cascade in response to certain stimuli in the brain. Little is known about the role of TLR4 in learning and memory functions. *Objectives:* The present study was designed to examine the involvement of TLR4 in olfactory-based spatial learning disability induced by exposure of environmental pollutants during the developmental period in neonatal mice. *Methods:* Pregnant C3H/HeN (TLR4 intact) and C3H/HeJ (TLR4 deficient) mice were purchased from Charles River Japan Inc. (Yokohama, Japan) and exposed to clean air, DE ($100 \pm 25 \mu g/m^3$) and DE-SOA ($120 \pm 25 \mu g/m^3$) from gestational day 14 to postnatal day (PND) 10 (5 h/day for 5 days) in exposure chambers. We have generated DE-SOA by oxidation of diesel exhaust particle with ozone. In the olfactory-based learning behavior, the time to reach the target goal cage was recorded for each PND 11 neonatal mouse using a video-assisted computer and Any-maze software (Muromachi Kikai, Tokyo, Japan). After completion of the spatial learning test, the hippocampus from each mouse was removed and examined for

the expressions of memory function-related genes and TLR4 signaling pathway genes using real-time RT-PCR. *Results:* Regarding the olfactory-based spatial learning test, both C3H/HeN and C3H/HeJ mice exposed to DE or DE-SOA took a longer time to reach the target as compared to respective control mice, but memory impairment was found greater in C3H/HeN mice compared to the C3H/HeJ mice. The expression levels of memory function-related genes such as the N-methyl-D aspartate (NMDA) receptor subunits NR1 and NR2B, and of TLR4 signaling pathway genes such as NFκB, TNF-α, COX2 and Iba1 were significantly increased in the hippocampi of the DE-SOA-exposed C3H/HeN newborn mice as compared to the control mice, but not in C3H/HeJ mice. *Conclusion:* We suggest that the potential toxic substances contained in DE-SOA may reach the brain via the systemic circulation or the olfactory nerve route to induce olfactory-based learning deficits in neonatal mice. Our results indicate that TLR4 may play an important role in environmental pollutant-induced neuroinflammation and spatial learning impairment by modulating the expressions of memory function-related genes and TLR4 signaling pathway genes in neonatal mice.

Disclosures: **T. Win Shwe:** None. **Y. Fujitani:** None. **H. Watanabe:** None. **S. Hirano:** None.

Poster

740. Transcriptional and Epigenetic Mechanisms of Learning and Memory

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Topic: H.01. Animal Cognition and Behavior

Support: JSPS Grant Scientific Research A 23240091

Human High Performance

Global Initiative for Sports Neuroscience

Title: Astaxanthin-enriched diet potentiates the effects of mild exercise on hippocampal-dependent memory and neurogenesis in adult mice

Authors: ***J. YOOK**, H. SOYA;
Advanced Res. Initiative For Human High Performace, University of Tukuba, Japan

Abstract: Recent studies have revealed that some dietary supplements, such as epicatechin and DHA, and exercise have similar roles in enhancing spatial memory and adult hippocampal neurogenesis (AHN). Their synergistic effects, however, have been shown only for memory, leaving some issues to be addressed before their clinical use. We recently reported that chronic mild exercise (ME) is sufficient to enhance both spatial memory and AHN (Inoue *et al*., PLoS

One, 2015), and that chronic administration of astaxanthin (ASX, 0.5%), a marine-organism-derived natural carotenoid, has the same effect on the hippocampus (Yook *et al*., Mol Nutr Food Res, 2016). Here, with the aim of developing clinically applicable methods, we postulated that ASX-enriched diets would improve memory by increasing AHN with special molecular mechanisms in adult mice. Male C57BL/6J mice (12 weeks old) were housed individually, and received ASX-enriched diets (0.5% ASX) with or without treadmill exercise (an intensity of below ventilatory threshold: 7 m/min) for 4 weeks. To evaluate spatial memory, the Morris water maze (MWM), including four days of acquisition and one day of probe tests, was performed in the final week of the intervention. To label newborn cells, all mice were intraperitoneally injected with 5-bromo-2'-deoxyuridine (BrdU, 100 mg/kg) for 3 consecutive days before the intervention. Immunohistochemistry was performed on coronal sections of the brain to examine AHN. The potential molecules underlying ME and/or ASX-induced AHN were analyzed using DNA microarray and Ingenuity Pathway Analysis database for genes identified in the hippocampus. The results of the MWM revealed that both ME and ASX produced significant increases in spatial learning and memory. The effects of ME on increased spatial memory were further enhanced when combined with ASX-enriched diets. The results of AHN analysis demonstrated that ME and ASX individually increased proliferation cells ($Ki67^+$) and newborn mature neurons ($BrdU^+/NeuN^+$), and their combination induced a greater increase in AHN than their respective individual effects. Using an omics approach, upregulation of genes associated with AHN, such as *Lep* and *Cxcr4*, was commonly observed in ME and ME+ASX. Interestingly, *Igf1r* was upregulated with the combination of ME and ASX, but not with ME alone. These results, which show that an ASX diet synergistically potentiates the ME effects on spatial memory associated with AHN with novelly identified molecular insights, support our hypothesis and intimate a powerful intervention strategy of combining ME and ASX for cognitive failure based on hippocampal structure and function. Thus we have begun clinical trials.

Disclosures: J. Yook: None. **H. Soya:** None.

Poster

740. Transcriptional and Epigenetic Mechanisms of Learning and Memory

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Evelyn F. McKnight Brain Research Foundation

NIH T35 Medical Student Summer Research Program

Title: Experience dependent epigenomic reorganization

Authors: *C. G. DUKE[1], A. KENNEDY[2], D. SWEATT[2], J. DAY[2];
[1]Sch. of Med., [2]Neurobio., UAB, Birmingham, AL

Abstract: The formation and maintenance of new memories requires transcription and translation of genetic material, and epigenetic mechanisms such as DNA methylation and demethylation serve as powerful regulators of this gene expression that is crucial to these processes. Moreover, aberrant DNA methylation has been identified in neurological and psychiatric disease states associated with impaired cognition, such as Alzheimer's disease, autism-spectrum disorders, schizophrenia, and drug addiction. Despite the clear necessity for epigenetic and transcriptional changes in memory formation, the precise nature of these phenomena has not been comprehensively explored. To illuminate this area, we harnessed whole-genome sequencing tools to systematically characterize memory-related changes in gene expression and DNA methylation status following memory acquisition. Using a hippocampus-dependent memory task (contextual threat learning), we report widespread and coordinated DNA methylation changes in the hippocampus (CA1) of Sprague-Dawley rats that are specific to threat learning and target genes involved in synaptic transmission and neuronal communication. We observed thousands of significant gene expression and epigenomic changes that are experience dependent, and these modifications were evident as early as one hour following the learning experience, becoming more marked and pronounced after twenty-four hours. Gene ontology term analysis showed that significantly hypomethylated genes were enriched for functional categories related to synaptic transmission. Additionally, we integrated these datasets with previously characterized epigenetic and transcriptional changes in brain disease states to provide a comprehensive resource to aid in the identification of memory-relevant therapeutic targets. Our results shed new light on the gene expression and methylation changes involved in memory formation suggesting that this process is dynamic and experience dependent, in addition to providing a roadmap for future work to identify therapeutic targets.

Disclosures: **C.G. Duke:** None. **A. Kennedy:** None. **D. Sweatt:** None. **J. Day:** None.

Poster

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Title: Interleukin-1 has a brain region- and memory task-specific role in heroin-conditioned contextual memory

Authors: *C. LEBONVILLE[1], L. HUTSON[1], M. JONES[1], J. PANICCIA[1], L. COOPER[1], R. FUCHS[2], D. LYSLE[1];
[1]Psychology and Neurosci., Univ. of North Carolina At Chapel Hill, Chapel Hill, NC;
[2]Integrative Physiol. and Neurosci., Washington State Univ., Pullman, WA

Abstract: Interleukin-1 (IL-1), a pro-inflammatory cytokine, has been linked to learning and memory. Specifically, this cytokine is thought to be involved in the maintenance of long term potentiation (LTP), a proposed mechanism of learning-related neuroplasticity. Studies also suggest that IL-1 is involved in hippocampal dependent, yet not hippocampal independent, memory. Previous studies in our lab have implicated IL-1 as having a role in a contextual immune conditioning paradigm using heroin. Like many other opiates, heroin suppresses the immune system. Similarly, exposure to contexts associated with heroin use can produce immunosuppression through Pavlovian conditioning. This learned effect relies on the learned association between a novel context and the immunosuppressive properties of opiates. The functional integrities of the dorsal hippocampus (DH), the basolateral amygdala (BLA), and the nucleus accumbens shell (NAcS) are necessary for this conditioned effect. Our laboratory demonstrated that siRNA-mediated IL-1β gene silencing in the DH prevents the *expression* of heroin-conditioned immunosuppression. Here, we expanded on this finding by 1) investigating the role of IL-1 in other brain regions shown to have a role in heroin conditioned immunosuppression and 2) expanding on its role within the DH using another context-heroin associative learning task. For the first goal, rats were conditioned to associate heroin with a distinct context. After training and 30 min before a test session, rats received bilateral microinfusions of the endogenous IL-1 receptor antagonist (IL-1RA) or saline into either the BLA, NAcS, or the caudate putamen (CPu) as a control region. Rats were then either re-exposed to the heroin-paired context (tested for expression of conditioned immunosuppression) or remained in their home cages (control) and their immune system was challenged using

lipopolysaccharide (LPS). Splenic iNOS and plasma nitrate/nitrite levels were assessed 6 h later. Disruption of IL-1 signaling in the BLA, but not the NAcS nor CPu, blocked the expression of heroin-conditioned immunosuppression. For goal two, rats were trained using a conditioned place preference (CPP) paradigm where one side of an apparatus was paired with heroin. After training and 30 min before testing, IL-1RA was infused into the DH yet this manipulation did not disrupt expression of CPP. Collectively, these studies demonstrate that the role of IL-1 in memory depends on the specific contextual memory task and which brain regions are under investigation.

Disclosures: C. Lebonville: None. **L. Hutson:** None. **M. Jones:** None. **J. Paniccia:** None. **L. Cooper:** None. **R. Fuchs:** None. **D. Lysle:** None.

Poster

740. Transcriptional and Epigenetic Mechanisms of Learning and Memory

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Topic: H.01. Animal Cognition and Behavior

Support: SATT AxLR maturation project

Title: Analysis of topographical memory and hippocampal neurogenesis in mice using the hamlet test

Authors: *L. CROUZIER, D. GILABERT, M. ROSSEL, F. TROUSSE, T. MAURICE; Inserm U1198, Montpellier, France

Abstract: The Hamlet test is an innovative behavioral analysis appliance that provides a complex environment following a resolutely anthropomorphic approach for testing topographical memory and spatio-temporal disorientation in mice. The apparatus mimics a small village with a central agora and streets expanding from it, leading to functionalized houses (Drink, Eat, Play, Run, Interact). Animals are habituated in groups of 6/8 individuals during 4 h a day, for several weeks. Memory can be tested by depriving mice from water (or food) during 20 h and testing, in a 10-min session, their ability to locate the Drink (or Eat) house. We analyzed the exploration pattern and topographical memory in different mouse strains (Swiss, C57BL/6, AKR) and gender. Habituated mice were compared to a control group of mice kept in room stabling. Significant differences in exploration patterns and memorization of the streets were observed between the strains and gender. The contribution of the hippocampus, which plays a central role in memory and topographical and spatial navigation, was investigated in C57BL/6 mice. Neurogenesis was analyzed in terms of proliferation, maturation and survival processes. First, we

observed an increase in proliferative and immature neuron markers, such as the increase in the number of Ki67 and doublecortin (DCX) positive cells in the subgranular zone of the dentate gyrus in the habituated group. Using 3D fluorescence imaging, longer and highly branched DCX positive neurons were visualized in the habituated group compared to non-habituated. In addition, newborn neurons were labeled using BrdU injection in the two groups and BrdU were revealed after 24 h to label newborn neurons or after 2 weeks to measure cell survival. The data obtained so far suggest that orientation and memorization in the Hamlet test promotes differentiation and maturation of adult neural progenitors and boosts neuroplasticity. Besides, neuroanatomical structures activated by habituation in the Hamlet were labeled using cFos and FosB and expression levels of memory-related immediate early genes are currently analyzed. Therefore, the Hamlet test allows the study of topographical memory in mice based on complex environment habituation and could provide an innovative tool to many research needs in various scientific fields.

Disclosures: **L. Crouzier:** None. **D. Gilabert:** None. **M. Rossel:** None. **F. Trousse:** None. **T. Maurice:** None.

Poster

740. Transcriptional and Epigenetic Mechanisms of Learning and Memory

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Topic: H.01. Animal Cognition and Behavior

Support: NIH R00 MH093459

Title: Systemic inflammation induces persistent dysregulation of memory and gene expression

Authors: ***D. TCHESSALOVA**[1], K. M. COLLETTE[2], A. A. KEISER[2], L. M. TURNBULL[2], A. E. WEHBE[2], N. C. TRONSON[2];
[2]Dept. of Psychology, [1]Univ. of Michigan, Ann Arbor, MI

Abstract: Systemic immune activation occurring during illness or surgery results in cognitive and memory deficits that last for years after recovery. To better understand how acute inflammatory signaling induces persistent alterations in memory, we used subchronic systemic immune challenge and examined long-lasting changes in memory, epigenetic modifications, and gene expression. There are sex differences in immune responses and susceptibility to persistent memory deficits, therefore we compared male and female mice throughout this study. Our paradigm consisted of intermittent systemic injections of one of two immune-inducing agents: the bacterial endotoxin lipopolysaccharide (LPS: 250ug/kg) or the viral analog polyI:C (6

mg/kg). Persistent changes in hippocampal-dependent memory were assessed either one week or eight weeks after the last injection using contextual fear conditioning and novel-object recognition. No alterations in hippocampal-dependent memory were observed at one week. In contrast, there was significant, sex-specific dysregulation of memory in both tasks eight weeks after the last injection. Notably, females, but not males, showed altered context fear conditioning. These findings demonstrate that alterations in memory emerge long after immune signaling has resolved, in a sex-dependent manner. Enduring changes in histone modifications and gene expression in the hippocampus were detected using western blotting and RNAseq, respectively. Of particular interest, were histone acetylation and methylation (eg. H3K9ac, H3K27me3) marks together with expression of neuroimmune proteins, neurotrophins, and stress-associated signaling pathways. Collectively, these data demonstrate that a systemic immune challenge induces sustained dysregulation of hippocampal-dependent memory and enduring differences in gene expression that may underlie the changes in memory. Systemic inflammatory events, such as major illness, cause long term alterations in epigenetic regulation and gene expression that contribute to the development of sex-specific memory impairments, cognitive alterations, and mood disorders.

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Poster

740. Transcriptional and Epigenetic Mechanisms of Learning and Memory

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Topic: H.01. Animal Cognition and Behavior

Support: 1ZIAMH002784

Title: The impact of neurogenesis on flexible maze training: effects on hippocampal volume, depression, and cognition

Authors: *T. J. SCHOENFELD, D. RHEE, H. A. CAMERON;
NIMH/NIH, Bethesda, MD

Abstract: Hippocampal volume loss is a biomarker for depression and is correlated with cognitive defects. Volume recovery is suggested to mediate symptom improvement, however little is known about the cellular changes responsible for hippocampal volume change. Research shows that ablation of neurogenesis in adult rats reduces hippocampal volume and blocks behavioral effects of antidepressants. Taxi drivers have larger hippocampi than non-drivers,

suggesting that spatial training induces hippocampal growth. Therefore, we were interested in devising a rat model of spatial training-induced hippocampal growth and exploring the role of neurogenesis in that growth. To do this, we created a novel maze, called the flex maze, with interchangeable walls that enables us to create multiple mazes within the same arena. We treated GFAP-TK transgenic rats (TKs) and WT littermates with valganciclovir for 8 weeks to specifically ablate adult neurogenesis. Rats were then trained on three different mazes, each with unique olfactory and visual cues, over the course of 4 weeks. Control rats also had daily access to the arena, but with only a fixed route towards reward not requiring any maze solving. After training, rats were tested on novelty suppressed feeding (NSF) to measure depressive-like behavior or an object location test to assess hippocampal-dependent memory. Brains were perfused and extracted to measure volume using a 14.1T MR scanner. Both WTs and TKs learned mazes at the same rate, showed spatial knowledge in probe trials, and retrieved mazes well after training. Only the maze scented with peppermint, an aversive odor for rats, showed a genotype difference, as WTs were slower to solve this maze and did not retain it in retrieval trials. Flex maze-trained WTs had larger hippocampi than controls, which was fully explained by increases in ventral CA1. TKs did not show this volume increase, but had smaller hippocampi than WTs, with significant volume decreases in the dentate gyrus and CA3. Both flex maze-trained WTs and TKs had less depressive-like behavior in NSF, but only flex maze-trained WTs showed increased learning in the object location test. The data suggest only 4 weeks of flex maze-training is sufficient to increase CA1 volume in ventral sections, however this is dependent on the presence of adult-born neurons in the hippocampus.

Disclosures: **T.J. Schoenfeld:** None. **D. Rhee:** None. **H.A. Cameron:** None.

Poster

740. Transcriptional and Epigenetic Mechanisms of Learning and Memory

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Support: MH81004

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P200A120165

GM055246

Title: Chemogenetic modulation of the medial habenula during cocaine action.

Authors: *A. J. LOPEZ, M. E. ESPINOZA, K. M. SAKATA, A. O. WHITE, J. L. KWAPIS, A. AL-KACHAK, Y. ALAGHBAND, D. P. MATHEOS, M. A. WOOD, 92697;
Dept. of Neurobio & Behavior; Ctr. for the Neurobio. of Learning & Memory, Univ. of California Irvine Dept. of Neurobio. and Behavior, Irvine, CA

Abstract: The medial habenula is an epithalamic region characterized by a dense projection to the interpeduncular nucleus. This projection is known as a hub for nicotinic acetylcholine receptors and cholinergic signaling. As such, addiction research has focused on the MHb with regard to nicotine, specifically nicotine withdrawal and self-administration. Yet, the role of the MHb in response to other drugs of abuse, such as cocaine, remains relatively uncharacterized and unclear. We use Designer Receptors Exclusively Activated by Designer Drug (DREADDs) to assess the role of the MHb in cocaine-associated memory. DREADDs are a novel tool that can be applied to drive changes at the cellular, circuit and, ultimately, behavioral levels. Their unique sensitivity to clozapine-n-oxide (CNO) paired with specific viral approaches allow for DREADDs to target particular brain regions, and more importantly, specific cell-types. Here, we demonstrate that the MHb is sensitive to specific cocaine treatments and is engaged by reinstatement of cocaine-induced conditioned place preference. We assessed the role of acetylcholine neurons in the MHb in relapse-like behavior using a conditioned place preference paradigm. ChAT-IRES-Cre mice received bilateral infusions of Cre-dependent excitatory (DIO-HM3D) or inhibitory DREADD (DIO-HM4D) into the MHb (0.5uL, M/L: +/- 0.33; A/P: -1.55; D/V: -3.0). Following conditioning and extinction, animals were primed with 3 mg/kg CNO (0.5% DMSO, 0.9% saline; I.P.) or vehicle (equal w/v) 40 minutes prior to being reinstated. For DIO-HM3D experiments, animals were reinstated with saline. For DIO-HM4D experiments, animals were reinstated with 5 mg/kg of cocaine-HCl. We demonstrate that DREADD-induced activation of MHb acetylcholine neurons can induce reinstatement of cocaine-induced CPP in the absence of cocaine. Conversely, DREADD-induced inhibition is capable of blocking cocaine-primed reinstatement of CPP. These experiments support previous work demonstrating the MHb is engaged following cocaine re-exposure. Moreover, in demonstrating the MHb is necessary and sufficient for reinstatement of cocaine-induced CPP, we provide evidence for its unique role in associative memory and other drugs of abuse.

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Poster

740. Transcriptional and Epigenetic Mechanisms of Learning and Memory

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Topic: H.01. Animal Cognition and Behavior

Title: Fatty acid amide hydrolase overexpression in the basolateral nucleus of the amygdala paradoxically decreases anxiety and reduces fear expression in rats

Authors: *M. MORENA[1], K. D. LEITL[2], A. RASHID[3], S. A. JOSSELYN[3], M. N. HILL[2]; [1]Cell Biol. & Anat. and Psychiatry, [2]Univ. of Calgary, Calgary, AB, Canada; [3]Univ. of Toronto, Toronto, ON, Canada

Abstract: The endocannabinoid (eCB) system is well known to play an important role in the regulation of emotional states and cognitive processes. A growing amount of evidence has demonstrated that elevations in the eCB anandamide (AEA) signaling within the basolateral complex of the amygdala (BLA) attenuate stress responses such as anxiety and fear expression. Specifically, inhibition of AEA hydrolysis by the enzyme fatty acid amide hydrolase (FAAH) within the BLA has been shown to reduce anxiety, neuroendocrine responses to stress and promote fear extinction. To determine if impairments in AEA signaling within the BLA would produce the opposite effects, and induce a stress-like state characterized by heightened anxiety and sustained fear, we examined the effects of overexpression of FAAH locally within the BLA on behavioural indices of anxiety and fear memory dynamics. Male adult Sprague Dawley rats were bilaterally infused in the BLA with an Herpes simplex virus type 1 vector, which preferentially infects principal neurons, containing FAAH and green fluorescent protein (HSV-FAAH-GFP) or a control vector containing only GFP (HSV-GFP). 72 h following HSV administration, a time point in which protein transfection is maximal, we found increased FAAH-mediated AEA hydrolysis together with decreased AEA levels within the BLA, confirming that the virus did successfully increase FAAH expression. At this same time point, a separate cohort of rats was tested for anxiety behaviour in an elevated plus maze, a light/dark box and an open field task. Quite surprisingly, we found that the overexpression of FAAH induced consistent anxiolytic effects in all three behavioural tasks we performed, relative to HSV-GFP rats. An additional cohort of animals was tested for fear memory extinction in an auditory fear conditioning paradigm. To prevent any effects of FAAH overexpression on the initial fear memory consolidation, animals were first fear conditioned and 24 h after conditioning, the animals were injected with HSV-FAAH-GFP or its control vector. 72 h following HSV administration rats were re-exposed to the tone alone for 4 consecutive days to examine fear extinction dynamics. Unexpectedly, rats infused with the HSV-FAAH-GFP vector exhibited a dramatic reduction in fear expression when exposed to the tone after conditioning, particularly on the first day of extinction training as compared to their HSV-GFP control rats.

These findings suggest that the exact modes of action of AEA within the amygdala in the regulation of emotional states and memory are still far from being clear, thus, opening the avenue to investigate new potential mechanisms by which these processes may occur.

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Poster

740. Transcriptional and Epigenetic Mechanisms of Learning and Memory

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Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant MH087463

Title: Nuclear receptor Nr4a mutant mice have impaired hippocampus dependent memory formation

Authors: ***S. CHATTERJEE**[1], W. O'BRIEN[2], M. BRIDI[3], J. HAWK[3], T. ABEL[1]; [1]Biol., [2]Dept. of Neuroscience,, [3]Neurosci. Grad. Group, Univ. of Pennsylvania, Philadelphia, PA

Abstract: New experiences are initially encoded as labile short-term memories, which are converted into stable long-term memory by gene transcription-dependent processes. Gene expression after learning involves a transient wave of transcription that is critical for memory consolidation. Following early transcriptional activity, learning also induces persistent long-lasting transcriptional changes that are reported to be involved in the storage of long-term memory. Increased transcript levels of 13 nuclear receptors, including all 3 members of the Nr4a subfamily, have been identified after learning. Interestingly, after learning, the promoters of Nr4a1, Nr4a2 and Nr4a3 show higher occupancies of histone H3.3, a mark for active transcription. In the present study, we show that transgenic expression of a dominant-negative form of NR4A (Nr4aDN) in forebrain neurons impairs long-term memory consolidation in spatial object recognition and Barnes maze. Importantly, the Nr4aDN mice do not show anxiety related behaviors in elevated zero maze. Golgi staining showed reduced dendritic spine density from CA1 pyramidal neurons of Nr4aDN mice. Quantification of the active components of AMPA and NMDA receptors at the synapses of Nr4aDN mice are being performed. We also identified Bdnf and Fosl2 as major targets of Nr4a dependent transcription. Chromatin Immuno-precipitation confirmed the binding of Nr4aDN to the promoters of Bdnf and Fosl2. Therefore,

the present study further underscores the importance of Nr4a function during hippocampus dependent memory storage.

Disclosures: **S. Chatterjee:** None. **W. O'Brien:** None. **M. Bridi:** None. **J. Hawk:** None. **T. Abel:** None.

Poster

740. Transcriptional and Epigenetic Mechanisms of Learning and Memory

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Topic: H.01. Animal Cognition and Behavior

Support: KAKENHI 25460077

Title: Comparative metallo- and element-omics profiling reveals aberrant copper contents in the brain of Ts1Cje, a mouse model for Down syndrome

Authors: ***K. ISHIHARA**[1,2], E. KAWASHITA[1], K. YAMAKAWA[2], H. SAGO[3], S. AKIBA[1]; [1]Kyoto Pharmaceut. Univ., Kyoto-Shi, Japan; [2]Lab. for Neurogenetics, RIKEN Brain Sci. Inst., Wako-shi, Japan; [3]Ctr. of Maternal-Fetal, Neonatal and Reproductive Med., Natl. Ctr. for Child Hlth. and Develop., Tokyo, Japan

Abstract: It is poorly understood the dyshomeostasis of elements including trace metals in the brain with Down syndrome (DS), although trace metals such as zinc, manganese, copper and iron are necessary for the growth and function of the brain. Since the Ts1Cje mouse is widely used as an animal model of DS, we examined here the brain levels of biogenic trace elements including biogenic metals in the brain of Ts1Cje mice. The levels of ultra-trace, trace, and major elements in the hippocampus, striatum, cerebral cortex, and cerebellum of Ts1Cje mice and wild-type littermates were assessed by inductively coupled plasma mass spectrometry (ICP-MS). Semi-quantification analysis revealed that the levels of some elements were disturbed in the cerebral cortex, hippocampus, striatum and cerebellum of Ts1Cje mice at 3 months of age (n = 7) compared with wild-type mice (n = 10). Especially, the concentration of copper ion in the brain of Ts1Cje mice was significantly increased in the three brain regions, cerebral cortex, hippocampus, and cerebellum. Furthermore, 1.5-2-fold increase in the concentration of copper ion in the Ts1Cje brain (n = 7) compared with wild-type mice (n = 6) was confirmed by a punctual quantitative analysis of ICP-MS. To explain the mechanisms of excess accumulation of copper ion in the brain of Ts1Cje, we assessed the expression of copper transporting proteins, Ctr1, Atp7a, and Atp7b. The P-type ATPases, Atp7a and Atp7b, both transport copper out of cells or into the trans-Golgi network, whereas Ctr1 is a plasma membrane protein that functions

as a high-affinity cellular copper uptake transporter. Immunoblot analysis revealed that the expression of Ctr1 was significantly decreased in the cerebral cortex, hippocampus, and cerebellum, but not in the striatum of Ts1Cje mice at 3 months of age (n = 3) compared with wild-type mice (n = 3). In contrast, Atp7a and Atp7b were expressed at a similar level in the brain regions of both genotypes. Our findings suggest that the dysregulated accumulation of copper ion may contribute a cognitive impairment in Ts1Cje mice.

Disclosures: K. Ishihara: None. **E. Kawashita:** None. **K. Yamakawa:** None. **H. Sago:** None. **S. Akiba:** None.

Poster

740. Transcriptional and Epigenetic Mechanisms of Learning and Memory

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 740.13/III16

Topic: H.01. Animal Cognition and Behavior

Support: NSERC 436204-2013

Dept. of Psychiatry Dalhousie University

Title: Maternal behaviour effects on atrx expression, genome stability and longterm neurobehavioral development in the adult offspring

Authors: *I. C. WEAVER, K. LEE, A. KORGAN, E. WAUGH, A. HUNDERT, E. MACRAE, D. GOGUEN;
Psychology & Neurosci., Dalhousie Univ., Halifax, NS, Canada

Abstract: Mounting evidence indicates that the maintenance of chromatin architecture is essential for normal human development and cognitive function. The ATRX gene, which is essential for normal growth and cognitive development, encodes a chromatinremodeling protein that is expressed in developing neural structures, including newlyborn cortical and hippocampal neurons in mice. Employing a new model of gestational psychological stress, in combination with wellestablished models of physical prenatal stress, we have shown that maternal behavior mediates the effects of gestational psychological stress on neural ATRX gene expression in the offspring, which is associated with alterations in DNA methylation, DNA damage signaling and stable individual differences in learning and memory and anxietyrelated and social behavior, as well as cortical and hippocampal function in the adult animal. These results and those from crossfostering studies support the possible involvement of ATRX in the sensitization of neurons to stress hormones and the programming of somatic behavior in response to maternal care. Since

disruption of ATRX impairs cognition and motor functions, inhibits learning in mouse pups, and contributes to developmental silencing of imprinted genes that shape somatic growth and brain, we hypothesize that the effects of motheroffspring interactions during the first week of postnatal life on ATRX expression and function influences both genome integrity and gene expression programs that underlie normal cognitive and emotional development. The elucidation of the mechanisms involved in the impact of neonatal nurturing addresses perhaps the most challenging question in development: How are experiences occurring in early life rendered permanent? In the case of genome stability and sustained changes in gene expression in brain cells, we can begin to understand the neurobiological basis for individual differences in personality and cognition as well as related risk for learning and behavioural disorders (e.g., Autism Spectrum, ADHD).

Disclosures: I.C. Weaver: None. **K. Lee:** None. **A. Korgan:** None. **E. Waugh:** None. **A. Hundert:** None. **E. Macrae:** None. **D. Goguen:** None.

Poster

740. Transcriptional and Epigenetic Mechanisms of Learning and Memory

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Topic: H.01. Animal Cognition and Behavior

Support: SFB 874 B2

stiftung mercator

Title: Modelling episodic memory deficit in depression

Authors: *J. FANG, S. CHENG;
Ruhr-University Bochum, Bochum, Germany

Abstract: Major depressive disorder (MDD) is a common and costly disorder associated with considerable morbidity, disability, and risk for suicide. MDD is characterized by a constellation of behavioral, emotional and cognitive symptoms especially in the domain of memory. In particular, many studies find an impairment of episodic memory in MDD patients, some observing an almost linear correlation between episodic memory performance and scores on a depression rating scale. Although the mechanisms underlying this memory dysfunction remain unclear, diverse animal models of depression find impaired adult neurogenesis (AN) in dentate gyrus. These newborn cells synaptically integrate into the hippocampal circuit and provide the potential substrates for new learning and memory. Both psychophysiological and computational studies suggest that dentate gyrus AN plays a critical role in minimizing interference between

overlapping memories by pattern separation, by which similar, but not identical, inputs are made less similar at the output stage. Therefore, we propose that inhibition of AN related to depression would prevent pattern separation so that the ambiguity between stored memories further impairs the accuracy of episodic memory retrieval. Here, we adapt a computational model of the semantic and episodic memory system, which we developed in previous work. In this model, a semantic representation is learned as a low dimensional representation of high-dimensional sensory input. During encoding of an episode, sequences of semantic representation patterns are stored as episodic memory. We model the impact of AN on episodic memory via its effect on pattern separation. In this model, AN adds additional noisy components to the original memory patterns. These components are unique to each memory sequence, i.e., patterns in the same sequences share the same noisy components, but they differ between different sequences. Hence, our model implements pattern integration between patterns within a sequence and facilitates discrimination between similar memory patterns in different sequences. This feature distinguishes our model from previous approaches, which assume that pattern separation operates on individual patterns. Our results show that retrieval performance is significantly better for the memories stored and retrieved during an asymptomatic phase, as compared to those memories stored and retrieved during a depressive episode. We also observe a retrograde effect for memories stored during an earlier asymptomatic phase, but retrieved during a later depressive episode.

Disclosures: J. Fang: None. **S. Cheng:** None.

Poster

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Program#/Poster#: 740.15/III18

Topic: H.01. Animal Cognition and Behavior

Title: Dimorphic modulation of the membrane-associated progesterone receptor neudesin in adult hippocampal neurogenesis

Authors: *A. NOVAIS[1,2], C. FERREIRA[2], F. MARQUES[2], N. SOUSA[2], J. PALHA[2], J. C. SOUSA[2];
[1]Life and Hlth. Sci. Res. Inst. (ICVS), Braga/Guimarães, Portugal; [2]Life and Hlth. Sci. Res. Inst. (ICVS), Sch. of Hlth. Sciences, Univ. of Minho and ICVS/3B's - PT Government Associate Laboratory,, Braga/Guimarães, Portugal

Abstract: Neudesin, also known as neuron derived neurotrophic factor (NENF), is a heme/steroid binding protein. *In vitro* it has been shown to be a potent stimulator of embryonic

neuronal precursors proliferation and differentiation, as well as a survival factor for neurons. Surprisingly, *in vivo* NENF's putative neurotrophic action has only been reported to be novel anorexigenic neurotrophic factor. Since NENF presents a cytochrome b5-like heme/steroid binding domain in it's primary structure, it is classified as a member of the membrane associated progesterone receptor family similarly to PGRMC1, a protein that binds progesterone to induce rapid non-genomic effects independent of nuclear receptors. Although NENF's binding to progesterone has not been proven yet, NENF protein structure has 39% of structural homology to PGRMC1 and has been hypothesized to participate in these cascades. In the brain, progesterone and its metabolites are reported to influence, with gender-specificity, cellular plasticity and neurogenesis particularly at the hippocampus. Another important characteristic of progesterone is its anxiolytic effect revealed to act through non-genomic actions. In this study, we used neudesin-null mice and performed a global characterization adult behavior, as well as, assessed the proliferation status of both adult neurogenic niches (hippocampus and sub ventricular zone). In adulthood neudesin-null males show an anxious-like phenotype in novel contextual exposure paradigms, as the elevated plus maze (EPM), light-dark box and the novelty suppress feeding tests. These gender differences were also present in the neurogenic niche of the hippocampus (sub granular zone) but not in the sub ventricular zone. Additionally neudesin-null males show an impaired freezing behavior in the contextual fear conditioning protocol, a task dependent on adult newly born neurons in the dentate gyrus. Neudesin-null mice gender differences together with the possible involvement of neudesin in the non-genomic actions of progesterone made us hypothesized if neudesin might be involved in this phenomenon. Our data might support a mechanistic link between progesterone, neudesin and hippocampal neurogenesis in the modulation of contextual dependent tasks.

Disclosures: **A. Novais:** None. **C. Ferreira:** None. **F. Marques:** None. **N. Sousa:** None. **J. Palha:** None. **J.C. Sousa:** None.

Poster

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Program#/Poster#: 740.16/III19

Topic: H.01. Animal Cognition and Behavior

Title: Proteomic analysis of intracellular signaling pathways underlying visual recognition memory in monkey

Authors: *B. A. CORGIAT[1,2], C. MUELLER[2], J. N. TURCHI[4], J. L. OLDS[3], R. C. SAUNDERS[4], L. A. LIOTTA[2], M. MISHKIN[5];

[1]NIMH/NIH, Bethesda, MD; [2]George Mason Univ., Manassas, VA; [3]George Mason Univ., Fairfax, VA; [4]NIMH, Bethesda, MD; [5]NIMH, Bethesda, VA

Abstract: Visual recognition memory is critically dependent upon the perirhinal cortex. More specifically, visual memory formation requires cholinergic activation of perirhinal M1 receptors and is characterized by enhanced multiunit activity in the upper middle and deep layers of perirhinal cortex. However, the M1 muscarinic-dependent intracellular signaling profiles underlying the critical synaptic changes induced during visual memory formation are unknown. Since the M1 receptor activates numerous intracellular pathways, we attempted a proteomic approach that allows for a multiplexed analysis of signaling proteins from numerous brain regions. Using reverse phase protein microarrays (RPPA), preliminary data from our group indicated (1) highly variable phosphorylation levels of M1-dependent proteins in perirhinal cortices and other medial temporal lobe structures when sampled across three monkeys, and (2) a time-dependent decrease in phosphorylation levels of M1 proteins after tissue extraction. As a result we modified our tissue harvesting protocol to include (i) freezing the tissue more rapidly, (ii) freezing bilateral areas simultaneously, and (iii) increasing the amount of tissue sampled per brain region. These modifications, plus a within animal interhemispheric comparisons, improve our ability to see protein changes related to memory function in future behavioral experiments.

Disclosures: B.A. Corgiat: None. **C. Mueller:** None. **J.N. Turchi:** None. **J.L. Olds:** None. **R.C. Saunders:** None. **L.A. Liotta:** None. **M. Mishkin:** None.

Poster

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Topic: H.01. Animal Cognition and Behavior

Support: INAIL-BRiC (ID:23; 2015) to LL

Title: Olfactory memory is enhanced via Wnt/β-catenin signaling in the subventricular zone of mice exposed to extremely low-frequency electromagnetic fields

Authors: *A. MASTRODONATO[1], S. BARBATI[1], L. LEONE[1], C. COLUSSI[2], K. GIRONI[1], C. GRASSI[1];
[1]Inst. of Human Physiol., Univ. Cattolica Med. Sch., Rome, Italy; [2]Inst. of Cell Biol. and Neurobiology, CNR, Monterotondo (RM), Italy

Abstract: We recently reported that exposure to extremely low-frequency electromagnetic fields (ELFEF) enhances hippocampal-dependent spatial learning and memory by increasing hippocampal neurogenesis. The present study was aimed to investigate whether ELFEF stimulation also affects olfactory memory by modulating neurogenesis in the subventricular zone (SVZ) of the lateral ventricle, and to identify the underlying molecular mechanisms.
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 ± 3.8 vs 64.2 ± 3.9%, respectively; n=8; $p<0.01$). Sixty minutes after the first trial, animals were exposed again to the same odor for 5 min: ELFEF-exposed mice showed a significant decrease in the time of exploration on the second presentation compared to controls (4.9 ± 0.5 [n=7] vs 9.9 ± 1.1 s [n=8], respectively; $p<0.001$) and an increased digging time near the odor that was previously associated to a reward (19.0 ± 1.4 vs 9.9 ± 0.9 s respectively; n=8; $p<0.001$).
Immunohistochemical analyses performed in ELFEF-exposed mice revealed increased NSC proliferation in the SVZ, as assessed by the number of $BrdU^+/Nestin^+$ cells (833.7 ± 0.9 vs 532.6 ± 0.1 in controls; n=3; $p<0.01$). Newborn NSCs differentiating towards the neuronal phenotype (i.e., $BrdU^+/DCX^+$ cells) were similarly increased in the rostral migratory stream of ELFEF-exposed mice (408.2 ± 1.1 vs 118.6 ± 0.7 cells in controls; n=3; $p<0.001$). This difference was retained when we counted the number of new cells that had become mature neurons in the olfactory bulb (i.e., $BrdU^+/NeuN^+$ cells) 30 days after the end of ELFEF stimulation protocol (560.2 ± 14.3 vs. 262.2 ± 14.0 in controls; n= 3; $p<0.001$). These effects were associated with upregulated expression of mRNAs encoding for critical regulators of adult neurogenesis, including Wnt3, and with an increased nuclear localization of the transcription factor β-catenin. *In vivo* injection of the Wnt3 inhibitor Dkk-1 (200 ng /0.5 µl) into the SVZ of ELFEF-exposed mice reverted ELFEF's effect on olfactory memory. Dkk-1 treatments of cultured NSCs inhibited the expression of neurogenic genes suggesting that the ELFEF-induced increase of olfactory memory relies on SVZ neurogenesis modulation.
Collectively, our findings unveil a new molecular mechanism regulating olfactory memory in mammals that relies on Wnt/β-catenin pathway and open the way to novel treatments of brain disorders associated with impaired adult neurogenesis and olfactory memory including neurodegenerative diseases and mood disorders.

Disclosures: **A. Mastrodonato:** None. **S. Barbati:** None. **L. Leone:** None. **C. Colussi:** None. **K. Gironi:** None. **C. Grassi:** None.

Poster

740. Transcriptional and Epigenetic Mechanisms of Learning and Memory

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Topic: H.01. Animal Cognition and Behavior

Support: Bial Foundation Grant - 253/14

Title: The impact of phospholipase D1 and D2 genetic ablation in hippocampal functioning

Authors: *T. G. OLIVEIRA[1], I. CASTANHO[1], L. S. MARINHA[1], R. R. SILVA[1], A. M. MIRANDA[1,2], F. V. BRAVO[1], G. DI PAOLO[2], V. PINTO[1];
[1]ICVS/3Bs, Univ. of Minho, Braga, Braga, Portugal; [2]Columbia Univ., New York, NY

Abstract: Over the past years increasing amount of attention has been given to signaling lipids as well as to its modulating enzymes, such as phospholipases. Specifically, phospholipase D (PLD), that converts phosphatidylcholine to phosphatidic acid, has been shown to exhibit a role in neurological development and physiology. Several studies have been associating PLD1 and PLD2, the two main mammalian PLD isozymes, to neurological processes, including neurotransmitter release, dendritic branching, cognition, and brain development. Also, the hippocampus has been suggested as one of the brain regions showing the highest PLD activity and neurodegenerative conditions such as Alzheimer's disease associated pathways have been shown to be modulated by PLD signaling. Thus, the aim of this project is to better understand the potential role of PLD in hippocampal function in adult mice upon Pld1 or Pld2 genetic ablation. We performed a hippocampal related behavioral characterization of these animals and a structural analysis regarding dendritic morphology. Our behavioral data, specifically considering motor and exploratory activity, anxiety and memory, showed that PLD2 knockout mice behavior is not altered when compared to their wild type littermates. Although most of this was also observed in the animals lacking PLD1, the results indicate an object recognition-dependent short-term memory deficit. Regarding hippocampal dendritic arborization, the ablation of either PLD1 or PLD2 led to an increase in dendritic morphology, although with a different impact of each isozyme in the dorsal and ventral hippocampus. Our results suggest the separate ablation of PLD1 and PLD2 leads to different effects in the dorsal and ventral hippocampus.

Disclosures: **T.G. Oliveira:** None. **I. Castanho:** None. **L.S. Marinha:** None. **R.R. Silva:** None. **A.M. Miranda:** None. **F.V. Bravo:** None. **G. Di Paolo:** None. **V. Pinto:** None.

Poster

740. Transcriptional and Epigenetic Mechanisms of Learning and Memory

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Topic: H.01. Animal Cognition and Behavior

Support: NIH grant MH068013

Title: Variations in intersession habituation behavior and genetic background within two cohorts of the same Il7r knockout mouse

Authors: *G. W. BOTHE[1], A. F. EISENER-DORMAN[1,2,3], D. J. SYMULA[1], V. J. BOLIVAR[1,2];
[1]Wadsworth Center, NYS Dept. of Hlth., Albany, NY; [2]Dept. of Biomed. Sciences, Sch. of Publ. Hlth., State Univ. of New York at Albany, Albany, NY; [3]Pharmaceut. Product Develop. LLC, Morrisville, NC

Abstract: Targeted ablation, one of the most commonly used tools in mouse genetics, plays an important role in elucidating the complexities of gene function. However, it is critical to remember that conventional gene ablation methodology has limitations that could lead to erroneous conclusions about the actual role of the targeted gene. Although a phenotype can be due to the absence of the targeted gene, it may instead be the result of residual embryonic stem cell-derived genetic material, either in the flanking region immediately surrounding the ablated gene or in the rest of the genetic background. The use of different strains/substrains for subsequent breeding can also introduce genetic variation that may contribute to an observed phenotype. To illustrate this point, we investigated the genetic underpinnings of a habituation behavior deficit using two cohorts of the same Il7r-/- knockout mouse obtained from The Jackson Laboratory. Using our published breeding scheme and whole-genome genotyping, we determined that the genetic background was responsible for the habituation deficit: the Il7r-/- cohort with a mixed C57BL/6J (B6J) and C57BL/6TB6/N (B6N) background and a greater amount of residual 129S7-derived genetic material exhibited poor habituation, whereas the Il7r-/- congenic cohort with a primarily B6/J genetic background had habituation levels similar to those of B6J controls. Further testing is necessary to determine whether the behavioral differences are due to residual 129S7-derived genetic material or genetic differences between the two B6 substrains; however, our findings underscore the importance of investigating the genetic background of knockout mice to avoid misinterpretation of results.

Disclosures: G.W. Bothe: None. **A.F. Eisener-Dorman:** None. **D.J. Symula:** None. **V.J. Bolivar:** None.

Poster

740. Transcriptional and Epigenetic Mechanisms of Learning and Memory

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Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant R01 MH102595

NIH Grant R00 MH083943

Title: Optogenetic interrogration of the role of dorsal and ventral hippocampal adult-born neurons in contextual fear conditioning

Authors: *K. A. HUCKLEBERRY[1], T. COPELAND[1], F. SHUE[1], W. YIN[2], R. A. CHITWOOD[1], M. R. DREW[1];
[1]Ctr. for Learning and Memory, [2]Div. of Pharmacol. and Toxicology, The Univ. of Texas At Austin, Austin, TX

Abstract: The hippocampus contains one of the few neurogenic niches within the adult brain—the subgranular zone of the dentate gyrus (DG)—and exhibits significant functional heterogeneity along its dorsoventral axis. Although adult-born neurons within the DG have been implicated in many hippocampus-dependent behaviors, little is known about how the function of the adult- born neurons varies along this axis. We used a highly specific Nestin-CreER(T2) mouse line (Lagace et al., 2007; Sun et al., 2014) to induce expression of the light-activated neural silencer archaerhodopsin in neural progenitor cells and their progeny. Optical fibers were implanted into the dorsal or ventral DG to selectively silence adult-born neurons in these regions. We then tested the contribution of ≤6-week-old adult-born granule cells to acquisition of context fear memory by delivering laser illumination during training. Neither dorsal nor ventral silencing affected the activity burst during the shock, indicating that silencing did not alter shock sensitivity. Silencing dorsal but not ventral adult-born neurons significantly decreased postshock freezing, a measure of short-term memory (Fanselow, 1980, 1982). Context memory expression was tested twenty-four hours after acquisition without laser illumination. Both dorsal and ventral silencing during acquisition were associated with reduced freezing in the context test. The data confirm earlier reports that adult hippocampal neurogenesis is required for acquisition of contextual fear (Saxe et al., 2006; Drew et al., 2010; Denny et al., 2012). However, the results conflict with other recent reports suggesting that ventral DG is not necessary for contextual fear conditioning (Khierbek et al., 2013; Danielson et al., 2016). Experiments in progress are assessing how inhibiting dorsal and ventral adult-born neurons alters local neural activity and the generalization of context fear. In summary, silencing dorsal adult-born neurons impaired both short- and long-term memory for context fear, whereas silencing the ventral adult-born neurons impaired only long-term memory. The data suggest that dorsal and ventral hippocampal adult-

born neurons make distinct contributions to context fear memory. We hypothesize that the dorsal adult-born neurons support context memory while the ventral adult-born neurons support the context-shock association.

Disclosures: K.A. Huckleberry: None. **T. Copeland:** None. **F. Shue:** None. **W. Yin:** None. **R.A. Chitwood:** None. **M.R. Drew:** None.

Poster

740. Transcriptional and Epigenetic Mechanisms of Learning and Memory

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Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant R00 MH093459 to NCT

Title: Inflammatory signaling and epigenetic modifications in persistent memory deficits after myocardial infarction

Authors: *K. COLLETTE[1], R. E. GARCÍA-HERNÁNDEZ[1], A. A. KEISER[1], D. TCHESSALOVA[2], L. M. TURNBULL[1], G. LU[1], N. C. TRONSON[1];
[1]Psychology, [2]Neurosci. Grad. Program, Univ. of Michigan, Ann Arbor, MI

Abstract: Cognitive impairment after an inflammatory event, such as myocardial infarction (MI) or major surgery, persists in many patients long after inflammation has abated. These cognitive deficits correlate with a higher rate of morbidity and mortality as well as reduced quality of life. Here we examined the mechanisms by which short-term inflammatory signaling causes persistent changes in neural function contributing to disruptions of memory. Prior results from our laboratory demonstrated that memory deficits and depression-like behavior occur 8 weeks after a cryo-injury model of MI in mice. In contrast, 2 weeks after MI, no memory impairments were evident. These findings suggest that the initial inflammatory event triggers ongoing molecular dysregulation that mediates persistent changes in affective processing and memory. To determine the interactions between neuroimmune signaling, intracellular signaling pathways, and epigenetic modifications leading to impaired memory, we examined specific changes in these factors over a time-course of 3 hours to 8 weeks following MI. We used MI, sham, and non-operated mice at each time-point. Cytokines (serum and hippocampus), MAPK signaling pathways, and histone acetylation in the hippocampus were examined shortly after surgery. At 1 and 8 weeks post-MI, we assessed memory and quantified levels of histone acetylation and methylation. After 8 weeks, we observed ongoing epigenetic changes including increased acetylation in male (H3K9) and female (H3-phosphoS10-acK14) hippocampus in a sex-specific

manner. Determining the mechanisms of persistent cognitive impairments after MI will identify novel targets for their treatment and prevention.

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Poster

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Topic: H.01. Animal Cognition and Behavior

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Evelyn F. McKnight Brain Research Foundation

Title: Extra-coding RNAs regulate dynamic DNA methylation and gene expression

Authors: *N. GALLUS, K. E. SAVELL, F. A. SULTAN, R. C. SIMON, J. S. REVANNA, J. J. DAY;
Univ. of Alabama at Birmingham, Birmingham, AL

Abstract: Epigenetic mechanisms in neurons are central regulators of neuronal function, experience-dependent gene expression, and adaptive behavior. DNA methylation is a well-studied epigenetic mechanism that exerts potent control over transcription and is critical for synaptic plasticity and long-term memory in multiple brain circuits. Although DNA methylation at specific sites in the genome is actively modified by neuronal activity and behavioral experience, it is presently unclear how methylation status at individual genes or even individual cytosine nucleotides can be targeted for modification. Extra-coding RNAs (ecRNAs) are non-coding, non-polyadenylated RNA species that arise from protein coding genes and regulate DNA methylation via direct interactions with DNA methyltransferases (DNMTs). Here, we used cortical neuronal culture systems to investigate the regulation, binding, and localization of a specific ecRNA transcript from the Fos gene locus. We find that this ecRNA is sensitive to multiple forms of neuronal activity, binds to both de novo and maintenance DNA methyltransferases with high affinity, and blocks DNA methylation at the Fos locus. To investigate the localization of ecRNA on a single cell basis, we employed single-molecule RNA FISH with multiplexed probes to separately identify mRNA and ecRNA transcripts. This technique confirmed activity-dependence of ecRNA induction and revealed a correlation between ecRNA and mRNA expression on a single cell level. Consistent with this observation,

anti-sense based knockdown of the Fos ecRNA selectively reduced Fos mRNA. Ongoing experiments are investigating the ability of targeted ecRNA delivery to specific gene loci to alter DNA methylation patterns and gene expression. Overall, these results suggest that ecRNAs are fundamental regulators of the establishment and perpetuation of DNA methylation patterns in neuronal systems, and reveal a promising avenue for epigenetic targeting in neurological and cognitive disease states.

Disclosures: **N. Gallus:** None. **K.E. Savell:** None. **F.A. Sultan:** None. **R.C. Simon:** None. **J.S. Revanna:** None. **J.J. Day:** None.

Poster

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Topic: H.01. Animal Cognition and Behavior

Support: CIHR

Title: CREB is not necessary for increases in hippocampal volume induced by environmental enrichment

Authors: ***D. A. VOUSDEN**, R. ALLEMANG GRAND, J. SCHOLZ, S. JOSSELYN, P. FRANKLAND, J. P. LERCH;
Neurosci. & Mental Hlth., Hosp. For Sick Children, Toronto, ON, Canada

Abstract: Human imaging studies show that experience leaves a lasting impact on brain structure. For instance, learning to juggle or navigate an environment alters the volume of specific brain regions (Zatorre 2012). However, the cellular mechanisms and signaling pathways underlying these volume changes remain poorly understood. Mouse imaging studies are ideal for probing the cellular bases of these changes. In rodents, environmental enrichment is a well-established paradigm to study the effect of experience on the brain (Diamond 1964). Environmental enrichment refers to housing conditions that provide more stimulation than standard cages, and can include toys, running wheels and social interaction. Enrichment is associated with many responses, including increases in dendritic branching, cell genesis and behavioural changes (van Praag 2000). Moreover, enrichment increases hippocampal volume at a scale detectable with mouse MRI (Scholz 2015). However the link between cellular and volume changes is unknown.
One hypothesis is that the enrichment effects are due to learning to navigate a novel, spatially complex environment, and are mediated by the same cellular mechanisms underlying learning

and long-term memory (LTM). Consistent with this, spatial maze-training also increases hippocampal volume in mice, but this effect is reduced in mice with impaired LTM. Given this, we hypothesized that enrichment-induced changes in hippocampal volume could be blocked by targeting the signaling pathways implicated in learning/LTM.
The transcription factor CREB is critical for LTM and modulates many processes stimulated by enrichment including dendritic growth. We used CREB deficient mice to ask whether volume changes induced by enrichment require CREB-dependent processes.
Methods: In vivo manganese-enhanced MRI was used to image adult CREB mutant mice (CREB+/+, CREB+/- & CREB-/-) at baseline, and then 48 hours, 1 week and 2 weeks after mice were placed into an enriched or standard environment. The enriched cage consisted of 3 storeys with tunnels and a running wheel. Automated image processing algorithms were used to detect volume changes associated with enrichment.
Results: Surprisingly, our preliminary results suggest that enrichment increases hippocampal volume regardless of CREB genotype. This implies that contrary to our hypothesis, the volume changes are mediated by CREB-independent pathways or that only residual CREB function is required. Future experiments will test our revised hypothesis that hippocampal volume changes induced by enrichment are mediated by CREB-independent effects of exercise, while those induced by spatial learning require CREB.

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Poster

740. Transcriptional and Epigenetic Mechanisms of Learning and Memory

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 740.24/III27

Topic: H.01. Animal Cognition and Behavior

Support: JSPS Research Fellowship for Young Scientists

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Title: Mapping emerging neocortical active ensembles during retrieval of remote memory via functional bioluminescence imaging of Arc promoter activity

Authors: *N. YAGISHITA-KYO[1], H. OKUNO[1,2], S. KAMIJO[1], T. KAWASHIMA[1], Y. UEMURA[3], S. TAKEMOTO-KIMURA[1,4], H. BITO[1];
[1]Grad. Sch. of Medicine, The Univ. of Tokyo, Tokyo, Japan; [2]Grad. Sch. of Medicine, Kyoto Univ., Kyoto, Japan; [3]Clin. Res. Support Center, The Univ. of Tokyo Hosp., Tokyo, Japan; [4]Res. Inst. of Envrn. Medicine, Nagoya Univ., Nagoya, Japan

Abstract: Activity-dependent gene expression is critical for the persistence of long-term memory. However, it has been challenging to identify, map and longitudinally compare active forebrain domains within the same animals *in vivo*, based on memory-related gene expression location during memory encoding and retrieval of remote memory. Previously, neurons activated after various cognitive tasks were determined *ex vivo*, based on immediate early gene (IEG) expression measured by means of *in situ* hybridization or serial two-photon tomography. To permit functional IEG imaging *in vivo*, we here developed a transcranial bioluminescence imaging system to enable widefield cortical recording of *Arc* promoter activity. First, we generated a transgenic mouse line in which an optimized luciferase reporter was driven under the control of the characterized full-length promoter of the *Arc* gene. We adopted a brighter version of luciferase (Emerald luciferase) as a reporter protein and further shortened its half-life using PEST sequence to minimize background and reliably measure *Arc* activation profile. In order to establish functional imaging from awake, head-fixed, intact-skull reporter mice, we further built an *in vivo* luminescence imaging system equipped with an EM-CCD, a head-fixed instrument, a light-proof box, and a continuous luciferin infusion port for long-term imaging. Using this imaging system, repeated transcranial imaging sessions were performed in the same animals over a couple of weeks, before, during and after an odor-reward association task, which initially required, but later became independent of, hippocampal activity. Based on image-based statistical analyses, we identified emerging, task-dependent, or memory retention period-dependent, increases in *Arc* promoter activity in several cortical areas associated with retrieval of remote memory of an odor-reward association task. These results shed new lights on the spatial spread and temporal dynamics of systems consolidation *in vivo*.

Disclosures: **N. Yagishita-Kyo:** None. **H. Okuno:** None. **S. Kamijo:** None. **T. Kawashima:** None. **Y. Uemura:** None. **S. Takemoto-Kimura:** None. **H. Bito:** None.

Poster

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Title: BDNF rescues BAF53b-dependent synaptic plasticity and cocaine-associated memory in the nucleus accumbens

Authors: *A. O. WHITE, E. KRAMÁR, A. LÓPEZ, J. KWAPIS, J. DOAN, D. SALDANA, F. DAVATOLHAGH, Y. ALAGHBAND, M. BLURTON-JONES, D. MATHEOS, M. WOOD; Neurobio. and Behavior, Univ. of California Irvine, Irvine, CA

Abstract: Recent evidence implicates epigenetic mechanisms in drug-associated memory processes. However, a possible role for one major epigenetic mechanism, nucleosome remodeling, in drug-associated memories remains largely unexplored. Here we examine mice with genetic manipulations targeting a neuron-specific nucleosome remodeling complex subunit, BAF53b. These mice display deficits in cocaine-associated memory that are more severe in BAF53b transgenic mice compared to BAF53b heterozygous mice. Similar to the memory deficits, theta-induced long-term potentiation (theta-LTP) in the nucleus accumbens (NAc) is significantly impaired in slices taken from BAF53b transgenic mice but not heterozygous mice. Further experiments indicate that theta-LTP in the NAc is dependent on TrkB receptor activation, and that BDNF rescues theta-LTP and cocaine-associated memory deficits in BAF53b transgenic mice. Together, these results suggest a role for BAF53b in NAc neuronal function required for cocaine-associated memories, and also that BDNF/TrkB activation in the NAc may overcome memory and plasticity deficits linked to BAF53b mutations.

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Poster

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Title: The role of HDAC3 in object location memory formation and the acquisition and extinction of cocaine-associated memory

Authors: *Y. ALAGHBAND, J. L. KWAPIS, R. DANG, O. V. AIMIUWU, A. AL-KACHAK, A. O. WHITE, A. J. LOPEZ, K. M. LATTAL, D. P. MATHEOS, M. A. WOOD; Neurobio. and Behavior and Ctr. for the Neurobio. of Learning & Memory, Univ. of California Irvine Dept. of Neurobio. and Behavior, Irvine, CA

Abstract: Chromatin modification is a molecular mechanism for both long-term memory formation and cocaine-induced neuroplasticity. Histone deacetylases (HDACs) are chromatin modifying enzymes that have been implicated as powerful negative regulators of memory processes. In these experiments, we investigate the role of HDAC3 (the most highly expressed Class I HDAC in the brain) in object-location memory as well as the acquisition and extinction of cocaine-associated memories using a genetic and viral approach. To investigate the role of HDAC3 in the dorsal hippocampus during object-location memory formation, C57BL/6J male mice received infusions of viruses which either overexpressed the *Hdac3* gene or had a dominant negative mutation of the gene. We demonstrate that impairing the enzymatic activity of HDAC3 in the dorsal hippocampus enhances object-location memory. Manipulating HDAC3 in the dorsal hippocampus also affects gene expression during the consolidation of object-location memory. Further, discrete stimuli or contexts previously associated with drugs of abuse are a major contributor to relapse among addicts. Conditioned place preference (CPP) is used to study cue-elicited context preference. Previous work from our laboratory has shown that inhibition of HDAC3 not only facilitates the acquisition of cocaine-CPP, but also modulates the extinction of drug-associated behavior in a manner that is subsequently resistant to reinstatement. The specific neural loci where HDAC3 plays this critical role in the acquisition and extinction of cocaine-cue memories is yet to be determined. In these experiments, we also explored the role of HDAC3 within the dorsal hippocampus and infralimbic cortex in cocaine-cue memories. Our findings demonstrate a critical role for HDAC3 in the molecular mechanisms underlying object-location and cocaine-cue memories.

Disclosures: Y. Alaghband: None. **J.L. Kwapis:** None. **R. Dang:** None. **O.V. Aimiuwu:** None. **A. Al-Kachak:** None. **A.O. White:** None. **A.J. Lopez:** None. **K.M. Lattal:** None. **D.P. Matheos:** None. **M.A. Wood:** None.

Poster

740. Transcriptional and Epigenetic Mechanisms of Learning and Memory

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Topic: H.01. Animal Cognition and Behavior

Support: Marsden Fund of the Royal Society of New Zealand

Title: Do adult-born granule cells "retire"?

Authors: *W. C. ABRAHAM[1,4,5,2], S. M. OHLINE[2,5,4], R. U. HEGEMANN[2,5,4], K. L. WAKE[2,5,4], M. F. DINNUNHAN[2,5,4], L. SCHWEITZER[2,5,4,3], S. M. HUGHES[3,5,4]; [2]Psychology, [3]Biochem., [1]Univ. of Otago, Dunedin, New Zealand; [4]Brain Res. New Zealand, Dunedin, New Zealand; [5]Brain Hlth. Res. Ctr., Dunedin, New Zealand

Abstract: Neurogenesis in the dentate gyrus occurs throughout adulthood in mammals. However, there is controversy whether adult-born neurons decline in their activity levels and eventually "retire" as recently born neurons functionally take their place in neural circuitry. To address this issue, we birth-dated neurons in the dentate gyrus of Sprague-Dawley rats at 35, 12, 6 and 4 wk prior to study at 10 months of animal age, using the thymidine analogues chloro-deoxyuridine and iodo-deoxyuridine. We used immunofluorescence to identify active neurons, defined as those showing co-localization of the thymidine analogue and the immediate early gene Zif268, as well as a neuronal marker, either calbindin or NeuN. We found that, as predicted, cells born at 12 and 6 wk before study were significantly less active (i.e. showed less Zif268 expression) than cells born 4 wk prior ($p = 0.002$ and $p = 0.01$, respectively). Surprisingly, the cells born 35 wk prior to study, when the animals were 2 months old, expressed Zif268 just as frequently as 4 wk old cells. We then asked whether the relatively young animal age (2 months) when these 35 wk cells were born was the reason for the retained activity level across time. Cells birth-dated at this animal age and studied 12 wk later (animal age 5 months) showed the same high level of Zif268 expression as cells that were 4 wk old in the same animals. This contrasted with the low activity reported above for cells born at 7 months and studied 12 wk later at 10 months, suggesting that the animal age at cell birth is a critical factor. To further test whether the 35 wk cell age was in some way special, we birth-dated cells at 35 wk and 12 wk (when the animal was 7 months and 12 months old, respectively) prior to study at 15 months. The levels of activity at these two time points were not significantly different from each other, but significantly lower than the activity of the 35 wk old cells born at 2 months of animal age at studied at 10 months ($p = 0.0037$). Thus the 35 wk cell age is not itself a determinant of cell activity. Instead, these results indicate that neurons born when a rat is young (2 months of age) continue to be highly active in neuronal circuitry throughout adulthood. If "retirement" does occur, it may be specific to cells born during an animal's middle-age.

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Poster

741. Cholinergic Modulation: Physiology and Behavior

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 741.01/III31

Topic: H.01. Animal Cognition and Behavior

Title: Cholinergic Modulation of pyramidal neurons in the ventral Subiculum

Authors: *S. WANG[1,2], D. TALMAGE[2,3], L. ROLE[1,2];
[1]Program In Neuroscience, SUNY At Stony Brook, Stonybrook, NY; [2]Neurobio. and Behavior, [3]Pharmacol. Sci., SUNY at Stony Brook, Stony Brook, NY

Abstract: The ventral Subiculum (vSub) is a major output area of the hippocampus with projections to multiple brain region related to the acquisition and retention of memories associated with emotionally salient experience. The vSub receives cholinergic input from the medial septum and diagonal band of Broca (MS-DBB) complex. Previous studies show that altering the cholinergic input to the vSub changes the output to other areas (e.g. basolateral amygdala, BLA; Calandreau et al., 2006), and can profoundly vSub involved acquisition and recall of fear memory (Kenny et al., 2012; Cox et al., 2013). However, the circuits that mediate these changes within the vSub are less well described and the mechanism by which acetylcholine (ACh) regulates the circuit remains unclear. This current study is to determine how ACh modulates the activity within the vSub circuit and to determine how ACh regulates the output of vSub pyramidal projection neurons (PYNs). To begin to assess the effect of ACh on regulating vSub PYN excitability in acute vSub slice preparations, we deliver ACh with or without the presence of ACh antagonists and monitor the membrane potential and evoked currents in individual PYN via patch-clamp. ACh (1mM or 10uM) increases the overall firing rate of vSub PYNs. Lower concentrations of ACh also elicit a transient decrease in the frequency of tetrodotoxin (TTX)-resistant inhibitory synaptic transmission (mIPSC) onto PYNs. The cholinergic modulation of mIPSCs is blocked by submicromolar atropine, consistent with a muscarinic ACh receptor (mAChR) mediated mechanism. ACh also elicits a transient increase in the frequency of TTX resistant excitatory synaptic transmission (mEPSC) onto PYNs, which may involve nAChRs as removal of nAChR antagonists also elicits a transient increase in mEPSC frequency. To examine the effects of cholinergic signaling in vSub circuit dynamics we have used genetically encoded calcium indicators to monitor the calcium level within vSub PYNs. ACh (1mM) induces a dramatic increase in calcium signaling in over 80% of neurons, as

well as a change in the extent of synchronization of calcium waves among neighboring neurons. These data are consistent with the idea that ACh can increase the excitability of PYN network in vSub, possibly via both mAChRs and nAChRs.

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Poster

741. Cholinergic Modulation: Physiology and Behavior

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Topic: H.01. Animal Cognition and Behavior

Support: NIMH Grant 5 F30 MH105087-02

Title: Alterations in the novel object recognition circuit following MeCP2 deletion from cholinergic neurons

Authors: *E. C. BALLINGER[1], C. P. SCHAAF[2], D. A. TALMAGE[1], H. ZOGHBI[2], L. W. ROLE[1];
[1]Stony Brook Univ., Stony Brook, NY; [2]Baylor Col. of Med., Houston, TX

Abstract: Rett Syndrome (RTT) is an autism spectrum disorder that affects approximately 1 in 20,000 girls and is caused by mutations in the gene encoding methyl CpG binding protein 2 (*MeCP2*). The cholinergic system appears to be particularly important in RTT, as decreases in cholinergic markers have been correlated with increased clinical severity in patients with RTT. Schaaf and Zoghbi have developed a powerful transgenic mouse model, whereby *MeCP2* is selectively deleted in cholinergic neurons only, to facilitate study of the contribution of this cholinergic lesion to the overall phenotype of RTT. Interestingly, this model exhibits a selective deficit in recognition memory, a form of declarative memory that has been shown by lesion and electrophysiological studies to be dependent upon cholinergic signaling in the perirhinal cortex (PRH). This memory deficit may map onto the intellectual disability seen in patients with RTT, however, its circuit level electrophysiological underpinnings are unknown. We use *in vivo* electrophysiology to compare baseline firing characteristics of PRH neurons in both mice in whom *MeCP2* has been selectively deleted in cholinergic neurons (*MeCP2* sKO) and control. We also use optogenetics to selectively activate cholinergic neurons in the Nucleus Basalis of Meynert, the cholinergic source nucleus that innervates the PRH, while simultaneously recording the effects of this selective activation to evaluate the cholinergic response profile of the PRH in each genetic condition. The network consequences of this altered responsiveness are evaluated using genetic markers of activity to map the recognition memory engram in both controls and

MeCP2 sKO. We have shown that PRH units of control are much more variable than PRH units from *MeCP2* sKO mice, perhaps indicating a loss of cholinergic modulation of firing. Additionally, the response of PRH units from *MeCP2* sKO to stimulation of cholinergic input is greatly reduced. Recognition memory engram mapping shows that while control mice show differential activation of the PRH when exposed to either a novel or a familiar object, *MeCP2* sKO mice show no such differential activation. The deficient cholinergic modulation of PRH unit firing that we have observed among *MeCP2* sKO mice may thereby impair encoding of the recognition memory engram and lead to the observed behavioral deficits. These results will further understanding of the role of ACh in encoding recognition memory and of the circuit level perturbations that may underlie cognitive phenotypes seen in patients with RTT.

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Poster

741. Cholinergic Modulation: Physiology and Behavior

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Topic: H.01. Animal Cognition and Behavior

Title: Cholinergic dysfunction of the entorhinal cortex in a mouse model of aging

Authors: *M. ANANTH[1], D. TALMAGE[2,3], L. ROLE[2];
[1]Program in Neurosci., [2]Dept. of Neurobio. and Behavior, [3]Dept. of Pharmacol. Sci., Stony Brook Univ., Stony Brook, NY

Abstract: Age-related cognitive decline is a growing problem; by the year 2050 over 80 million people in the United States will be aged 65 and older. As such, our understanding of the pathophysiology and progression of age-related cognitive decline is of the utmost importance. The cholinergic system has long been implicated in cognitive decline, and perhaps more specifically, severe pathological cognitive decline as observed in Alzheimer's disease. What remains unknown is the role of acetylcholine (ACh) in the temporal dynamics of age-related cognitive decline. The entorhinal cortex (EC) is an important relay station to the hippocampus, as well as a number of cortical areas. Importantly, it has been found to be particularly vulnerable in aging. The EC gets a major cholinergic projection from the basal forebrain (BF) cholinergic nuclei - a group of cholinergic nuclei that provide ACh to many cortical and subcortical structures. As the EC is affected early in aging, the circuit between the BF and the EC is of particular importance, and can give valuable information about how altered cholinergic system integrity in aging can alter cortical function. We have to our advantage a mouse model that

recapitulates aging pathology of both amyloid beta plaque and neurofibrillary tangle accumulation at an earlier physical age. Using this model, we use optogenetic stimulation and *in-vivo* electrophysiology to investigate the responsiveness of EC units to increased endogenous ACh.
Preliminarily, we have found that the health and integrity of the BF cholinergic nuclei are compromised in the aging model. When examining the extent of cholinergic innervation to the EC, we find that the aging model has less cholinergic terminals, and those that remain appear fragmented. Using *in-vivo* electrophysiology to understand the implications of these changes, we found that EC opto-stimulation differentially modulates EC units in the aging model as compared to controls. In response to increased ACh in the EC, control animals have an increase in firing rate, while aging animals have a change in the firing pattern with no increase in firing rate. Interestingly, at baseline, EC units in the aging model have elevated firing rate as compared to controls. These preliminary findings suggest that impaired cholinergic innervation of the EC may result in an elevated EC firing rate, which renders EC units unable to respond normally to endogenous ACh release. These studies can provide explanations as to the early ACh dependent vulnerabilities in EC function in aging. Additionally, these experiments provide key information regarding the circuit level implications that result from altered responsiveness to ACh.

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Poster

741. Cholinergic Modulation: Physiology and Behavior

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Topic: H.01. Animal Cognition and Behavior

Title: Amino acid metabolizing enzyme, l-amino acid oxidase, controls the contextual memory through modulating acetylcholine level

Authors: *K. USUDA, G. WATANABE, K. NAGAOKA;
Vet. Sci., United Grad. Sch. of Vet. Science, Gifu, Tokyo, Japan

Abstract: Amino acids participate directly and indirectly in a variety of important biochemical functions in brain. Recently, we identified that one of amino acid metabolic enzyme, L-amino acid oxidase (LAO), expressd in mice hippocampus. It has been known that LAO converts particular l-amino acids, phenylalanine, methionine and leucine etc. into keto acids, ammonia and hydrogen peroxide. In this study, we investigated the importance of LAO in brain function using by LAO knockout (KO) mice.
Firstly, we observed the presence of LAO protein in hippocampus of wild-type (WT) but not in

LAO KO mice by immunofluorescence analysis. In addition, we measured the concentration of free amino acids in the hippocampus using mass spectrometry analysis. It was found that some free amino acids such as phenylalanine significantly increased in the hippocampus of LAO KO mice compared to that of WT mice. Next, we conducted several behavior test, open field test, elevated plus maze test and passive avoidance test, and found that LAO KO mice showed higher anxiety and weaker short and long term contextual memory compared to WT mice. To understand the change of major neurotransmitter concentrations in the hippocampus, we also measured acetylcholine, noradrenaline, serotonin and dopamine by mass spectrometry analysis. Interestingly, acetylcholine concentration in LAO KO hippocampus was significantly lower than in WT. Thus, we administered donepezil, inhibitor of acetylcholinesterase, to LAO KO mice and conducted passive avoidance test. It should be noted that administration of donepezil clearly improved contextual memory in LAO KO mice. We also confirmed that the concentration of acetylcholine in LAO KO hippocampus after donepezil administration was recovered to same level in WT mice.
In conclusion, we revealed first evidence that LAO plays an important role in brain function, especially contextual memory through modulating acetylcholine level in hippocampus. Although we did not show exact mechanism how LAO modulates acetylcholine level, we provided one possibility that accumulation of phenylalanine might be related to decrease of acetylcholine level in LAO KO hippocampus.

Disclosures: **K. Usuda:** None. **G. Watanabe:** None. **K. Nagaoka:** None.

Poster

741. Cholinergic Modulation: Physiology and Behavior

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Topic: H.01. Animal Cognition and Behavior

Support: NSERC

Title: Role of the cholinergic midbrain in long-term habituation

Authors: A. LOUTTIT, N6G 1R3, E. AZZOPARDI, T. ZAMAN, *S. SCHMID;
Univ. of Western Ontario, London, ON, Canada

Abstract: Sensory filtering refers to the processes that allow us to pre-attentively filter out redundant or unnecessary sensory information and consists of one of the most basic forms of learning, habituation. Habituation is a progressive decrease in response following repeated exposure to a stimulus and can be studied using the acoustic startle response (ASR) and

exploratory behaviour in an open field as behavioural models. Habituation can be a either a transient process occurring within a single test session or long lasting when a decrease in response is observed across several sessions. The traditional view of cholinergic modulation of habituation is that acetylcholine is involved in habituation of motivated behaviour, such as exploratory behaviour, but is not involved in habituation of reflexive behaviour, such as the ASR. However, a recent study suggests that acetylcholine may be involved in long-term habituation of ASR. A potential cholinergic structure that has been previously implicated in modulating the startle response and locomotor behaviour is the pedunculopontine tegmental nucleus (PPT). We hypothesized that cholinergic PPT neurons modulate long-term habituation of the ASR and exploratory behaviour. To test the role of cholinergic PPT neurons in habituation, transgenic (CreChAT) rats were injected with an inhibitory DREADD (rAAV8-hSyn-DIO-hM4Di-mCherry) or control (rAAV8-hSyn-DIO-mCherry) viral construct. Long-term habituation of the ASR and exploratory behaviour was tested over five consecutive days in which a systemic injection of the DREADD activator clozapine-*N*-oxide (CNO) was delivered prior to the test session. Preliminary findings suggest that cholinergic PPT neurons are not involved in long-term habituation of the ASR, but may be involved in long-term habituation of exploratory behaviour. In addition to behavioural experiments, *in vitro* electrophysiology was performed to confirm neuronal silencing following CNO application.

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Poster

741. Cholinergic Modulation: Physiology and Behavior

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Topic: H.01. Animal Cognition and Behavior

Title: Reversal of scopolamine impairment by a type II nAChR alpha7 positive allosteric modulator in the NHP CANTAB paired associates learning task

Authors: ***M. R. WEED**[1], J. POLINO[1], D. POST-MUNSON[1], M. BOOKBINDER[1], L. KISS[2], J. HERRINGTON[1], L. J. BRISTOW[1];
[1]Genetically Defined Dis. and Genomics, [2]Leads Discovery & Optimization, Bristol-Myers Squibb Res., Wallingford, CT

Abstract: Performance on the human CANTAB paired associates learning (PAL) task is impaired in patients with mild cognitive impairment (MCI) and further impaired in Alzheimer's disease (AD). Indeed, worsening of PAL performance in MCI patients reliably predicts conversion to AD. Previous studies have demonstrated that scopolamine impairs performance on

a nonhuman primate (NHP) version of the CANTAB PAL task. Agonists at the nicotinic alpha 7 receptor subtype (nAChRa7) have been shown to significantly reverse scopolamine's impairment of the NHP CANTAB PAL task, and the present study investigated whether a positive allosteric modulator (PAM) selective for nAChRa7's would have a similar effect. nAChRa7 PAMs have been described as type I (where current is increased without changing receptor kinetics) and type II (receptor desensitization is slowed leading to a longer and larger charge transfer). Compound 1 potentiated peak ACh-induced currents 1-fold relative to a maximal ACh concentration 3 mM and slowed channel desensitization, resulting in a 52-fold increase in charge transfer, consistent with a type II nAChRa7 PAM. Cynomolgus monkeys (N=7) were trained to perform the CANTAB NHP PAL task, including multiple levels of task difficulty. Similar to previous reports, scopolamine pretreatment impaired NHP PAL performance in a difficulty-dependent fashion. Scopolamine-induced impairments were significantly ameliorated by Compound 1 at 0.1 mg/kg. These results are the first demonstrating scopolamine reversal by an nAChRa7 PAM in NHPs and are consistent with previous results where donepezil and nAChRa7 agonists significantly reversed scopolamine impairment of the NHP PAL task. Efficacy of nAChRa7 PAM in reversing scopolamine impairment is consistent with their development as therapeutics for cognitive decline in MCI or AD. In addition, the high degree of similarity of the NHP CANTAB PAL task and the human CANTAB PAL task provides a unique opportunity to perform highly translational research.

Disclosures: **M.R. Weed:** A. Employment/Salary (full or part-time): Bristol-Myers Squibb. **J. Polino:** A. Employment/Salary (full or part-time): Bristol-Myers Squibb. **D. Post-Munson:** A. Employment/Salary (full or part-time): Bristol-Myers Squibb. **M. Bookbinder:** A. Employment/Salary (full or part-time): Bristol-Myers Squibb. **L. Kiss:** A. Employment/Salary (full or part-time): Bristol-Myers Squibb. **J. Herrington:** A. Employment/Salary (full or part-time): Bristol-Myers Squibb. **L.J. Bristow:** A. Employment/Salary (full or part-time): Bristol-Myers Squibb.

Poster

741. Cholinergic Modulation: Physiology and Behavior

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Topic: H.01. Animal Cognition and Behavior

Support: Supported by the National Research Council of Science & Technology (NST) grant by the Korean government (MSIP) (No. G15120 and CRC-15-04-KIST)

Title: Anti-amnesic effects of Terminalia chebula extract on scopolamine-induced learning deficits in mice

Authors: *M.-S. KIM[1,2], J. LEE[1,2], J.-S. HAN[3], S. SUNG[4], H. KIM[4], D. LEE[4], W. JEON[1,2]; [1]KM Convergence Res. Div., Korea Inst. of Oriental Med., Daejeon, Korea, Republic of; [2]Korea Inst. of Sci. and Technol., Seoul, Korea, Republic of; [3]Konkuk Univ., Seoul, Korea, Republic of; [4]Seoul Natl. Univ., Seoul, Korea, Republic of

Abstract: *Terminalia chebula* extract (TCE) has been widely used for cardiotonic, diuretic, and homeostatic treatments in many Asian countries. The major components of TCE such as tannic acid and chebulagic acid have anti-oxidant, anti-mutagenic, and anti-inflammatory properties. Although several *in vitro* studies have examined acetylcholinesterase (AChE) inhibitory activities of TCE, its neuropharmacological effects on memory function remain to be examined. The present study, therefore, investigated the anti-amnesic effects of TCE using scopolamine model of memory impairments. Mice was orally administered with TCE (200 mg/kg) or donepezil (5 mg/kg) for 14 days (days 1-14), and scopolamine (1 mg/kg) was injected intraperitoneally for 7 days (days 8-14), 30 min before the Morris water maze task (MWM) which was used to examine cognitive status. The brain of animals was dissected to measure expression levels of AChE and choline acetyltransferase (ChAT) in the hippocampus. Animals with administration of scopolamine alone showed impairments in acquisition and retention in MWM and increased hippocampal AChE expression levels. TCE treatments significantly attenuated scopolamine-induced memory impairment and restored hippocampal AChE expression levels. In addition, TCE treatments increased hippocampal ChAT expression levels. These findings suggest that TCE might be cognitive enhancer associated with modulation of cholinergic neurons. Supported by the National Research Council of Science & Technology (NST) grant by the Korean government (MSIP) (No. G15120 and CRC-15-04-KIST).

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Poster

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Program#/Poster#: 741.08/III38

Topic: B.07. Synaptic Transmission

Support: Welcome rust - DBT Trust Alliance

Title: Neuromodulatory effects on acetylcholine on alpha rhythm generation and how it relates to cognitive functioning

Authors: *R. SHARMA[1], P. RAMAKRISHNA[2], S. NADKARNI[2];
[1]Biol., IISER-PUNE, Pune, India; [2]IISER-Pune, Pune, India

Abstract: Oscillations are said to play a vital role in organizing neuronal computations across various anatomical regions of the brain. These well characterized; wide range of brain rhythms are typically coordinated based on the intrinsic electrical properties of the neurons, network motifs, synaptic properties and neuromodulatory influences.
Acetylcholine(Ach) functions as a neurotransmitter, as well as a neuromodulator in the extra-cellular-matrix(ECM) driving activity in entire populations of neurons. In the thalamo-cortical circuit, the neuromodulatory effects of Ach can crucially change network excitability leading to activity in the alpha spectrum (one of the dominant oscillations of the brain).
In a biophysically detailed neuronal network model of the thalamo-cortical circuit, we explore the generation of alpha-rhythms and its mechanism of disruption in Alzheimer's Disease(AD). We quantify the detailed current dynamics of the thalamo-cortical circuit involved in alpha that leads to emergence of the rhythm via enhanced acetylcholine activity. We show how heterogeneity in gap-junctional coupling strength between high-threshold thalamocortical cells (HTCs) can produce rich dynamics wherein cells can fire in and out of phase in agreement with experiments and influence the alpha-rhythm activity. Further, we show how shifting alpha band to lower frequencies contributes to enhanced inhibitory drive to the HTC cells and can be the causal link to diminished power in the alpha spectrum seen in Alzheimer's patients. We also investigate how changes in intracellular calcium signaling related to AD can disrupt Alpha.

Disclosures: **R. Sharma:** None. **P. Ramakrishna:** None. **S. Nadkarni:** None.

Poster

741. Cholinergic Modulation: Physiology and Behavior

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 741.09/III39

Topic: B.07. Synaptic Transmission

Title: Regulation of the temporoammonic pathway in the hippocampus by acetylcholine

Authors: *J. PALACIOS[1,2], J. R. MELLOR[1,2];
[1]Physiology, Pharmacol. and Neurosci., [2]Ctr. for Synaptic Plasticity, Sch. of Physiology, Pharmacol. and Neurosci., Univ. of Bristol, Bristol, United Kingdom

Abstract: The release of acetylcholine in the hippocampus during awake behavior is important for encoding memory. Within the hippocampal network, acetylcholine has diverse effects which are not ubiquitous and instead are exhibited at individual neurons and synapses. The Temporoammonic (TA) pathway carries spatial information from grid cells in entorhinal cortex layer III (EC LIII) to CA1 hippocampal place cells synapsing onto the distal dendrites. It is not currently known how acetylcholine regulates synaptic transmission in the temporoammonic pathway or which acetylcholine receptors mediate this regulation. To determine how acetylcholine regulates the temporoammonic pathway we made whole cell patch clamp recordings from CA1 pyramidal neurons in acute hippocampal slices from adult mice. Electrical stimulation in the Stratum Lacunosum Moleculare was used to isolate excitatory postsynaptic currents (EPSC) in the presence of Picrotoxin and recording at -70 mV or disynaptic inhibitory postsynaptic currents (IPSC) recording at 0 mV. The acetylcholine receptor agonist carbachol (CCh 10 μM) reduced both excitatory and inhibitory synaptic responses and increased paired-pulse ratio for excitatory responses, indicating a presynaptic locus of action. Specific pharmacological intervention showed that neither M1 agonist was able to reproduce CCh induced synaptic currents reduction, nor M1 antagonist blocked the effect. In contrast, M3 receptor antagonist occluded CCh induced reduction of synaptic probability of release to the same extent for EPSC and IPSC. The reduction in synaptic response for excitatory and inhibitory responses was similar for both but the increase in paired pulse ratio for excitatory responses produced a facilitation of excitatory-inhibitory balance in response to repetitive stimulation. In addition, CCh produced an increase in the number of spikes in the CA1 pyramidal neurons when TA synapses were repeatedly stimulated over a range of frequencies. This increase was principally mediated by a membrane potential depolarization, rather than a synaptic effect. We conclude that acetylcholine modulates the temporoammonic pathway onto CA1 pyramidal neurons by presynaptically located M3 muscarinic receptors.

Disclosures: **J. Palacios:** None. **J.R. Mellor:** None.

Poster

741. Cholinergic Modulation: Physiology and Behavior

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 741.10/III40

Topic: B.07. Synaptic Transmission

Title: Microanalytical system for neural response to neurotransmitter using voltage imaging and electrochemical micropump

Authors: *K. HARADA, Y. YOSHIMI;
Shibaura Inst. of Thechnology, Tokyo, Japan

Abstract: “Brain-on-chip (BOC)” devises, where neurons are incubated in microchips simulating internal condition of brain, are feasible for study of the mechanism of disease or chemotherapy of brains or neurons. Response of neurons to neurotransmitters is an important factor to evaluate activity of neurons in disease or therapy. Then we developed a new BOC devise which uses an electrochemically driven diaphragm micropump for neurotransmitter administration and voltage imaging for detecting neural response which is less invasive to the chip than traditional electrophysiological method. The micropump was fabricated by attaching polydimethylsiloxane (PDMS) sheets using plasma-etching. Electrolysis of the electrolyte pushed diaphragm to jet out the neurotransmitter solution in the pump. Then neurons isolated from abdominal ganglion of *Aplysia californica* were allowed to adhere on poly-L-lysine coated PDMS sheet to incubate for 3-4 days. The neurons were stained with Di-4-ANEPPS as a voltage sensitive dye (VSD). The dyed neuron was placed facing to the nozzle of the pump. Neural response was detected by the fluorescent intensity of the VSD. The VSD imaging of the neurons detected the action potentials 481 ± 64 ms later than the voltage application to the pump in order to administrate 1 mM acetylcholine solution. But the administration of 3 M potassium chloride solution by the micropump, the action potential was detected with latency of 277 ± 26 ms after the voltage application to the pump. The difference of response time is consistent with the fact that high concentration of potassium ion changes membrane potential directly but acetylcholine induces the potential change through acetylcholine receptor on the membrane. The result indicates that the administration of neurotransmitter by the micropump is effective for measurement of neural response latency. Neural signal can be detected less than one second, this system may monitor the neural function in the presence of therapeutic drugs or toxins. In this new system, neurons are not attached to the electrode and can be stimulated by plural neurotransmitters. The micropump has a very simple structure, and then many micropumps can be integrated in a small chip, which is promising to elucidate mechanism of neuron in abnormal condition.

Disclosures: K. Harada: None. **Y. Yoshimi:** None.

Poster

741. Cholinergic Modulation: Physiology and Behavior

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Program#/Poster#: 741.11/III41

Topic: B.07. Synaptic Transmission

Support: NIH Grant MH099054

Title: Presynaptic control of glutamatergic transmission by serotonin and acetylcholine in the prefrontal cortex

Authors: *A. L. BAKER, A. T. GULLEDGE;
Geisel Sch. of Med. at Dartmouth, Lebanon, NH

Abstract: Neocortical pyramidal neurons comprise two broad classes of projection neurons: commissural/callosal (COM) neurons that provide interhemispheric connectivity, and corticofugal neurons that project to many subcortical targets, including, in the case of corticopontine (CPn) neurons, the pontine nuclei. COM and CPn neurons have distinct morphological and physiological characteristics, including divergent postsynaptic responses to the neuromodulators acetylcholine (ACh) and serotonin (5-HT). Despite the well-characterized selectivity of postsynaptic regulation of COM and CPn neurons by ACh and 5-HT, little is known about the presynaptic selectivity of these transmitters on glutamatergic transmission in the cortex. To examine this, we are expressing channelrhodopsin-2 (ChR2) in select afferent pathways to the medial prefrontal cortex, and testing presynaptic effects of ACh and 5-HT on light-evoked synaptic events in pairs of physiologically-identified COM and CPn neurons. We first characterized the synaptic connectivity of ChR2-expressing COM afferents in one hemisphere onto pairs of COM and CPn neurons in the contralateral hemisphere. Interhemispheric COM afferents targeted COM and CPn neurons with equal probability, and generated excitatory postsynaptic potentials (EPSPs) of similar magnitude in both neuron types across a range of light intensities (n = 19 pairs). However, across all light intensities, COM inputs to CPn neurons were more likely to initiate action potentials than were COM inputs to simultaneously recorded COM neurons ($p < 0.05$). Bath application of 5-HT (40 μM, in the presence of blockers of postsynaptic 5-HT_{1A} and 5-HT_{2A} receptors) reduced the amplitude of COM-evoked EPSPs in both COM (from 8.9 ± 1.1 to 4.5 ± 0.7 mV) and CPn (from 8.7 ± 1.0 to 3.5 ± 0.6 mV) target neurons (n=19 pairs; $p < 0.05$). Although reductions in EPSP amplitudes were somewhat larger in CPn neurons (-62 ± 3%; n = 25) compared to COM neurons (-49 ± 4%; n = 23; $p < 0.05$), this may reflect a decrease in input resistance (-6.3 ± 2.3%; $p < 0.05$) observed selectively in CPn neurons, which may result from insufficient blockade of 5-HT_{1A} receptors, or from the expression of additional postsynaptic 5-HT receptors in CPn neurons. Preliminary results suggest that endogenous ACh also potently reduces glutamate release from COM afferents onto contralateral COM and CPn target neurons. These results contribute to our understanding of the connectivity and neuromodulatory control of cortical circuits.

Disclosures: **A.L. Baker:** None. **A.T. Gulledge:** None.

Poster

741. Cholinergic Modulation: Physiology and Behavior

Location: Halls B-H

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Program#/Poster#: 741.12/III42

Topic: B.07. Synaptic Transmission

Title: Characterization of [^{3}H]PT-0921, a novel muscarinic acetylcholine receptor positive allosteric modulator radioligand selective for the M_4 subtype

Authors: *D. L. SMITH[1], C. R. BUTLER[2], R. E. O'CONNOR[3], M. POPIOLEK[1], L. ZHANG[2]; [1]Neurosci., Pfizer Inc, Cambridge, MA; [2]Neurosci. Medicinal Chem., Pfizer Inc., Cambridge, MA; [3]Pharmacokinetics, Dynamics, and Metabolism, Pfizer Inc., Groton, CT

Abstract: The availability of a potent and selective radioligand that binds to the M_4 muscarinic acetylcholine receptor (mAChR) allosteric site will facilitate the understanding of M_4 positive allosteric modulator (PAM) pharmacology. Herein, we profile a M_4 selective PAM radioligand, [^{3}H]PT-0921, in rat and human M_4 stable cell lines and native tissue assays. PT-0921 is a novel M_4 mAChR PAM shown to decrease cAMP production with an EC_{50} of 6.09 ± 1.46 nM (human) and 1.81 ± 0.13 nM (rat) for stably expressing M_4 HEK293 cells, measured using a potentiation assay in the presence of an EC_{20} concentration of acetylcholine (ACh). This receptor-selective ligand showed no functional PAM response at the M_1, M_2, M_3, and M_5 mAChR subtypes when tested up to 10 μM. Also, the effects on the affinity of ACh at the [^{3}H]NMS orthosteric binding site was examined in M_4 stable cell lines and PT-0921 reduced the concentration of ACh required to inhibit [^{3}H]NMS binding to the M_4 mAChR, left-shifting the EC_{50} of ACh by 35-fold (rat) and 65-fold (human) at 10 μM. Binding of [^{3}H]PT-0921 to coronal sections of the rat brain in autoradiographic localization studies showed distribution in the cortex, nucleus accumbens, caudate putamen, and olfactory tubercle, while being displaced by 10 μM of the M_4 PAM, Compound A. One limitation of PT-0921 is that it is a Pgp/BCRP substrate and though it gets into the brain, it is actively transported out. This liability made a [^{3}H]PT-0921 in vivo receptor occupancy assay unfeasible. To overcome this challenge, we took advantage of the Pgp/BCRB triple knockout mouse. In these transgenic mice we were able to utilize [^{3}H]PT-0921 for quantification of in vivo occupancy of another M_4 PAM, Compound B, which showed 92% receptor occupancy in the striatum when administered at 10 mg/kg. Taken together, these data show PT-0921 to be a selective M_4 PAM and [^{3}H]PT-0921 is shown to be a useful tool in the investigation of M_4 PAMs both in vitro and in vivo.

Disclosures: D.L. Smith: None. **C.R. Butler:** None. **R.E. O'Connor:** None. **M. Popiolek:** None. **L. Zhang:** None.

Poster

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Topic: B.07. Synaptic Transmission

Support: NIH Grant R01-MH104638

Title: Muscarinic modulation of GABAergic neurotransmission in the basolateral amygdala.

Authors: *A. FAJARDO SERRANO, L. LIU, D. D. MOTT, A. J. MCDONALD;
Dept. of Pharmacology, Physiol. and Neurosci., Univ. of South Carolina Sch. of Med., Columbia, SC

Abstract: The density of the cholinergic innervation of the basolateral nucleus of the amygdala (BL) by the basal forebrain is much greater than that of the neocortex or hippocampus. Activation of muscarinic receptors in BL is critical for the consolidation of many types of memory including inhibitory avoidance and fear conditioning. Since suppression of GABAergic inhibition is important for synaptic plasticity in the BL, it is essential to determine which, if any, interneuronal (IN) subpopulations are modulated by activation of distinct presynaptic muscarinic receptors. To investigate this question we performed paired recordings from presynaptic INs and postsynaptic PNs using whole-cell patch-clamp techniques in rat amygdalar slices. Putative INs and PNs were initially identified on the basis of differences in perikaryal size and shape, and electrophysiological properties. Application of muscarine (10 μm) suppressed the IPSCs evoked by presynaptic INs in the paired PNs by 80%, but left spontaneous IPSCs intact. In other studies, in both rats and mice, evoked monosynaptic IPSCs in PNs were inhibited by 50% by muscarine. This suppression was almost entirely reversed by the M2R antagonist AF-DX 116 (1 μm). The inability of muscarine to completely inhibit the IPSCs at saturating concentration suggests that M2Rs are present on only a subpopulation of inhibitory axon terminals. As in the cortex there are two main types of INs in the BL. INs expressing parvalbumin (PV) provide most of the perisomatic innervation of PNs, but also innervate PN dendrites. INs expressing somatostatin (SOM) mainly innervate distal dendrites and spines of PNs. Dual-labeling electron microscopic studies using immunoperoxidase and silver-enhanced immunogold procedures are now in progress to determine whether M2Rs are expressed in the axon terminals of PV+ and/or SOM+ INs. In addition, structures that are postsynaptic to these terminals are identified on the basis of morphological criteria. Limited preliminary data from M2R/PV preparations suggest that some PV+ terminals forming synapses with dendritic shafts may express M2Rs. Further analysis is required to confirm these findings, to gather data on PV inputs to PN perikarya, and to investigate expression of M2Rs in SOM+ axon terminals. In conclusion, electrophysiological and neuroanatomical findings to date suggest that activation of M2Rs in axon terminals of one or

more subpopulations of BL INs is critical for the disinhibition of PNs by ACh. This disinhibition could contribute to the consolidation of emotional memory by the BL.

Disclosures: **A. Fajardo Serrano:** None. **L. Liu:** None. **D.D. Mott:** None. **A.J. McDonald:** None.

Poster

741. Cholinergic Modulation: Physiology and Behavior

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Topic: B.07. Synaptic Transmission

Support: VA Merit 1I01BX001374

NIH Grant R01 MH104638

Title: Cholinergic signaling in fear extinction

Authors: ***G. C. JONES**, J. R. MCELROY, S. C. TRYON, A. C. SHARKO, M. A. WILSON, D. D. MOTT;
Dept. of Pharmacology, Physiol. and Neurosci., Univ. of South Carolina Sch. of Med., Columbia, SC

Abstract: Post-Traumatic Stress Disorder (PTSD) is a mental health disorder that can occur following a traumatic event such as being in combat, witnessing or being assaulted, or experiencing a natural disaster. Individuals with PTSD are unable or impaired in their ability to extinguish fear memories, resulting in disabilities preventing them from living normal, healthy lives. However, it remains unclear why some individuals exposed to traumatic events develop PTSD while others are more resilient and do not. Acetylcholine is known to play a critical role in fear learning, but its role in fear extinction is not as well understood. This study utilized a rat model of fear extinction to determine if individual differences in extinction learning are correlated with markers of cholinergic signaling. Cholinergic markers include M1 muscarinic acetylcholine receptor (M1 mAChR) and vesicular acetylcholine transporter (vAChT). These cholinergic markers are strongly expressed in brain regions, such as the amygdala and prefrontal cortex (PFC), which function in the fear extinction circuit. The goal of the present study was to determine whether individual differences in cholinergic signaling in these brain regions underlie the differences observed in fear extinction. Expression levels of cholinergic markers were measured in the amygdala and PFC of male Long-Evans rats after undergoing a Pavlovian fear conditioning and extinction paradigm. We found that rats exhibited individual differences in

extinction of freezing behavior following twenty presentations of a conditioned auditory stimulus. When M1 mAChR expression in these animals was assessed, a significant correlation was evident between expression level of M1 mAChR in the amygdala and the freezing behavior during the extinction trials. Expression of M1 mAChRs in the amygdala of animals showing good extinction learning was significantly higher than that in animals resistant to extinction. In contrast, there was no significant correlation between vAChT expression and freezing in either amygdala or PFC. These results suggest that low expression of M1 mAChRs in the amygdala is correlated with deficits in fear extinction. These results are in agreement with the role of muscarinic receptors in other aversive learning paradigms, and suggest that therapeutic strategies aimed at enhancing muscarinic signaling in the amygdala may enhance fear extinction in animals and perhaps patients with PTSD.

Disclosures: **G.C. Jones:** None. **J.R. McElroy:** None. **S.C. Tryon:** None. **A.C. Sharko:** None. **M.A. Wilson:** None. **D.D. Mott:** None.

Poster

741. Cholinergic Modulation: Physiology and Behavior

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Topic: B.07. Synaptic Transmission

Support: NIH Grant R01 MH104638

Title: Muscarinic receptors differentially regulate glutamatergic pathways in the basolateral amygdala

Authors: ***S. C. TRYON**, L. LIU, A. J. MCDONALD, D. D. MOTT;
Dept. of Pharmacology, Physiol. and Neurosci., Univ. of South Carolina Sch. of Med., Columbia, SC

Abstract: The basolateral amygdala (BL) is essential for emotional memory and fear learning. The BL receives strong recurrent excitation as well as afferent glutamatergic inputs from a variety of brain regions, including midline thalamic nuclei (Thal), prelimbic area (PL) of the medial prefrontal cortex and the lateral nucleus of the amygdala (LA). The BL is also the target of extensive cholinergic innervation from the basal forebrain. Acetylcholine acting in the BL plays a critical role in consolidation of fear memory, although exactly how it modulates synaptic transmission to BL is relatively unknown. To investigate this question we examined muscarinic acetylcholine receptor (mAChR) modulation of synaptic transmission at different glutamatergic inputs to the BL. Using optogenetics and whole cell electrophysiology in rodent amygdalar

slices, we compared the effects of mAChR activation with muscarine (10 μM) on synaptic transmission at recurrent and afferent inputs to BL pyramidal neurons (PNs). Bath application of muscarine strongly suppressed evoked EPSCs at PL inputs, while EPSCs at Thal inputs were only partially inhibited and input from LA was not inhibited. Muscarine also failed to inhibit recurrent excitation in BL. At cortical inputs muscarine-evoked inhibition was partially blocked by the M1 mAChR antagonist, telenzepine, and fully blocked by the M3 antagonist 4-DAMP, indicating that the inhibition was produced by activation of M1 and/or M3 mAChRs. Interestingly, while muscarine suppressed cortical input to BL PNs during a single stimulus, it increased the reliability of excitatory transmission during a stimulus train by reducing synaptic depression. This effect was greatest at stimulus frequencies in the gamma band (30-90 Hz). These findings suggest that during periods of high cholinergic tone mAChRs would filter cortical and thalamic input to BL, while leaving inputs from LA and recurrent inputs from BL unchanged. This may increase the signal to noise ratio for LA input to BL, allowing the LA to more strongly influence BL PNs at frequencies below gamma band. However, at gamma frequencies cortical inputs would be strengthened. This frequency dependent regulation could protect the BL from asynchronous afferent input, while increasing functional coupling and synaptic plasticity between BL and PL cortex.

Disclosures: **S.C. Tryon:** None. **L. Liu:** None. **A.J. McDonald:** None. **D.D. Mott:** None.

Poster

741. Cholinergic Modulation: Physiology and Behavior

Location: Halls B-H

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Program#/Poster#: 741.16/III46

Topic: B.07. Synaptic Transmission

Title: The atypical antipsychotic olanzapine disturbs depotentiation and reversal learning by modulating mAChRs

Authors: W. SONG, J.-H. CHA, S. YOON, *M.-H. KIM;
Seoul Natl. Univ. Col. of Med., Seoul, Korea, Republic of

Abstract: Olanzapine (Olz), one of the most frequently prescribed atypical antipsychotics, is generally considered a first-line drug for treating schizophrenia. In contrast to psychotic symptoms, the effects of Olz on cognitive symptoms of schizophrenia are still unclear. In addition, the mechanisms by which Olz affects the neural circuits associated with cognitive function are unknown. Here we investigated the effects and mechanisms of Olz on synaptic plasticity and the cognitive behavior of mice. Olz interrupts depotentiation without disturbing *de novo* LTP and LTD. At hippocampal SC-CA1 synapses, co-activation of NMDARs (N-methyl-

D-aspartate receptor), mGluRs (metabotropic glutamate receptors), and mAChRs (muscarinic acetylcholine receptors) is necessary and sufficient to reverse stably expressed LTP. Olz inhibits the activation of mAChRs, which amplifies glutamate signaling. Behaviorally, Olz impairs spatial reversal learning of mice in the Morris water maze test. Our results uncover a novel mechanism underpinning the cognitive modulation of Olz and show that the anticholinergic property of Olz affects glutamate signaling and synaptic plasticity.

Disclosures: W. Song: None. **J. Cha:** None. **S. Yoon:** None. **M. Kim:** None.

Poster

741. Cholinergic Modulation: Physiology and Behavior

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Topic: H.01. Animal Cognition and Behavior

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Title: Bursting cholinergic neurons of the basal forebrain show synchronous activity in an auditory detection task

Authors: *T. LASZLOVSZKY[1], A. KEPECS[2], B. HANGYA[1,2];
[1]Lendulet Lab. of Systems Neuroscience, IEM H, Budapest, Hungary; [2]Cold Spring Harbor Lab., Cold Spring Harbor, NY

Abstract: The cholinergic basal forebrain (CBF) has been implicated in diverse cognitive functions through its influence on cortical information processing. Earlier studies differentiated tonic and phasic effects of the CBF based on the varying timescales of cortical acetylcholine release (Parikh et al., 2007). In accordance, *in vitro* experiments described an early- and a late firing cholinergic group, likely corresponding to bursting and non-bursting neurons (Unal et al., 2012), proposing that bursting neurons convey phasic information while non-bursting neurons set tonic levels of cortical acetylcholine. However, this theory has not been tested *in vivo*, therefore it remains unclear how bursting and non-bursting groups of CBF contribute to

cholinergic effects at different time scales (Hangya et al., 2015).
To address this question we analyzed optogenetically identified and putative cholinergic neurons in the nucleus basalis (NB) (n = 44) and in the horizontal limb of the diagonal band of broca (HDB) (n=12) in mice performing an auditory detection task requiring sustained attention. Our analysis of autocorrelations (Royer et al., 2012) uncovered three types of cholinergic neurons: 'tonic' neurons showing long refractory periods, 'phasic bursting' neurons showing classical bursting phenotype and 'phasic non-bursting' neurons exhibiting short refractory but no prominent burst shoulders. By analyzing cross-correlations of concurrently recorded pairs of NB neurons (n = 16) we discovered that the bursting ones were synchronously active at a short timescale, unlike the tonically active cholinergic neurons. However, all three cell types were capable of fast and precise responses to behaviorally salient events, which clearly distinguished them from tonically active cholinergic interneurons of the striatum. Thus, bursts of cholinergic neurons likely reflect strong bottom-up excitatory drive that, with the added synchrony, leads to stronger and more wide-spread cortical activation.

Disclosures: **T. Laszlovszky:** None. **A. Kepecs:** None. **B. Hangya:** None.

Poster

741. Cholinergic Modulation: Physiology and Behavior

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Program#/Poster#: 741.18/III48

Topic: B.07. Synaptic Transmission

Support: CONACyT CB-2013-01 221653

Title: Smoking-like nicotine levels increase excitatory synaptic response in the prefrontal cortex of the mouse

Authors: *R. D. CUEVAS OLGUIN[1], F. VAZQUEZ-PONCE[1], E. ESQUIVEL-RENDON[1], J. VARGAS-MIRELES[1], I. GONZALEZ-NATERAS[1], M. MIRANDA-MORALES[1], H. ARIAS[2], M. ATZORI[1];
[1]UASLP, San Luis Potosi, Mexico; [2]Med. Educ., California Northstate Univ. Col. of Med., Elk Grove, CA

Abstract: The legal addictive drug nicotine produces multiple biological effects. Central effects of nicotine are still scarcely understood. Nicotine users report an emotional relief during the first several minutes following the intake of the drug by smoking. This phenomenon has been correlated with the co-release of several neurotransmitters, mainly dopamine, throughout the limbic system. In the present work we investigated the possibility that nicotine directly affects

synaptic transmission in the limbic system. We did so by determining the effect of a dose of nicotine equivalent to smoking one cigarette (300 nM) on the excitatory synaptic transmission of the prefrontal cortex - a brain region involved in the perception of pleasure and relief - in a murine model (C57BL/6). In order to determine the effect of nicotine on excitatory synaptic transmission, we performed patch-clamp recording on layer 5 visually-identified neurons of the medial prefrontal cortex, measuring electrically evoked pharmacologically isolated post-synaptic currents (eEPSC) in the presence of the blocker of γ-amino butyric acid (GABA) type A receptor ($GABA_AR$) blocker bicuculline meth-chloride. α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic receptor ($AMPA_R$)-mediated synaptic signals were recorded at the holding voltage V_h = -70 mV and measured as peak amplitude of the corresponding synaptic inward current, while N-methyl-D-aspartate receptor (NMDAR)-mediated currents were recorded at the holding voltage V_h = +40 mV and measured as the late current at a fixed interval of 180 ms after the stimulation artefact (in order to minimize the AMPAR-mediated contribution), both of them using a paired pulse protocol at 180 ms interpulse interval, every 12 s. Nicotine increased both AMPAR-mediated as well as NMDAR-mediated eEPSCs (120 ± 33 %, n = 8; 41 ± 22 %, n = 6, respectively), without changing the pair pulse ratio (ratio between the second response, A_2 and the first response A_1, PPR = A_2/ A_1) for either AMPAR-mediated eEPSC (0.95± 0.02 in control, vs. 0.83 ± 0.96) or NMDAR-mediated eEPSCs (0.79 ± 0.06 in control vs 0.73 ± 0.07 in nicotine, same samples). Our results show that the presence of nicotine acutely enhances glutamatergic synaptic transmission. The simultaneous increase of AMPAR-mediated and NMDAR-mediated signal, together with the lack of change in PPR, suggest that nicotine may increase excitatory synaptic response through a presynaptic mechanism, possibly through the increase in glutamate release. The identification of the type of nicotinic receptors involved in the enhancement of glutamatergic response and its physiological significance still require further investigation.

Disclosures: R.D. Cuevas Olguin: None. **F. Vazquez-Ponce:** None. **E. Esquivel-Rendon:** None. **J. Vargas-Mireles:** None. **I. Gonzalez-Nateras:** None. **M. Miranda-Morales:** None. **H. Arias:** None. **M. Atzori:** None.

Poster

741. Cholinergic Modulation: Physiology and Behavior

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Topic: B.07. Synaptic Transmission

Support: Okinawa Institute of Science and Technology Graduate University

Title: Cholinergic modulation of striatal projection neurons activity *In vivo*

Authors: *S. ZUCCA, A. ZUCCA, J. WICKENS;
Neurobio. Res. Unit, Okinawa Inst. of Sci. and Technol., Okinawa, Japan

Abstract: The striatum is the main input nucleus of basal ganglia. The principal output neurons of the striatum (striatal projection neurons, SPNs) transform the input to the striatum, originating from all areas of the cerebral cortex, into output activity that is transmitted to other basal ganglia structures. This transformation involves interactions of glutamatergic inputs from the cortex, with local input from other SPNs, extrinsic modulation by dopaminergic afferents, and local interneurons. Acetylcholine is released in high concentrations by a population of cholinergic interneurons (CINs), but the function of acetylcholine in the striatum remains enigmatic. We have recently shown that loss of cholinergic function in the striatum is associated with a significant impairment in learning of new behavioral strategies (Aoki et al., J Neurosci, 2015). However, how CINs firing pattern contribute to local striatal activity *in vivo* remains to be elucidated. To investigate this question, we made electrophysiological recordings in anesthetized mice from SPNs while optogenetically controlling CINs firing. CINs were identified *in vivo* by juxtacellular recordings and showed typical spontaneous firing with regular, irregular, and bursting patterns. SPNs exhibited low spontaneous firing frequency. We found that optogenetic activation or inactivation of CINs did not produce significant increase or decrease of their firing rates. We hypothesize that CINs may have a state-dependent modulatory effect on slow membrane oscillations of SPNs. To test this hypothesis, we will obtain whole-cell recordings from SPNs and compare the effect of CINs pause burst firing patterns on spontaneous membrane potential fluctuations and in responses to glutamatergic synaptic input.

Disclosures: S. Zucca: None. **A. Zucca:** None. **J. Wickens:** None.

Poster

741. Cholinergic Modulation: Physiology and Behavior

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Program#/Poster#: 741.20/III50

Topic: B.07. Synaptic Transmission

Title: Identifying novel genes required for synaptic function among human 21st chromosome orthologs

Authors: *S. K. NORDQUIST[1], J. PIERCE-SHIMOMURA[2];
[1]Inst. for Neurosci., The Univ. of Texas at Austin, Austin, TX; [2]Univ. of Texas at Austin, Austin, TX

Abstract: Trisomy of the 21st chromosome results in Down syndrome, one of the most common congenital disorders with an underlying genetic basis. Though Down syndrome is associated with many phenotypes, those that affect the nervous system present the greatest obstacles to achieving high quality of life. To better understand the genetic basis of neurological phenotypes in Down syndrome, we systematically screened all putative human 21st chromosome orthologs in *Caenorhabditis elegans* using both RNA interference (RNAi) and loss-of-function mutant analysis. In addition to screening for superficial neuronally dependent behaviors (e.g. locomotion), we also assessed neuromuscular synaptic function using the acetylcholinesterase inhibitor aldicarb. We identified several genes with decreased cholinergic secretion, including *mtq-2*, a highly conserved ortholog of the human 21st chromosome gene *N6AMT1*. Further, we determined a preliminary neuronal expression pattern for *mtq-2* in *C. elegans*. These data suggest a potential role of *mtq-2* in mediating nervous system function in worm and provide a platform on which mammalian researchers may build.

Disclosures: S.K. Nordquist: None. **J. Pierce-Shimomura:** None.

Poster

741. Cholinergic Modulation: Physiology and Behavior

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 741.21/III51

Topic: H.01. Animal Cognition and Behavior

Title: Oleanolic acid ameliorates scopolamine-induced cognitive impairment in mice

Authors: S. JEON, J. ZHANG, Y. GWON, *J. RYU;
Kyung Hee Univ., Seoul, Korea, Republic of

Abstract: Oleanolic acid is a naturally occurring triterpenoid that is widely present in food and medicinal plants. To examine the effect of oleanolic acid on memory deficits, we employed a scopolamine-induced cognitive deficit mouse model. A single administration of oleanolic acid significantly increased the latency on the passive avoidance task and affected the alternation behavior on the Y-maze task and the exploration time on the novel object recognition task, indicating a recovery of the cognitive impairment induced by scopolamine. In accordance with previous reports, oleanolic acid enhanced ERK1/2 and CREB phosphorylation and BDNF expression in the hippocampus. Together, these results imply that oleanolic acid ameliorates scopolamine-induced memory impairment by modulating the ERK1/2-CREB-BDNF pathway in mice, suggesting that oleanolic acid would be a potential therapeutic agent for the treatment of cognitive deficits.

Disclosures: **S. Jeon:** None. **J. Zhang:** None. **Y. Gwon:** None. **J. Ryu:** None.

Poster

741. Cholinergic Modulation: Physiology and Behavior

Location: Halls B-H

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Topic: H.01. Animal Cognition and Behavior

Title: Paradoxical effects of alpha 7 nicotinic acetylcholine receptor antagonists on memory formation and receptor functioning

Authors: ***N. P. VAN GOETHEM**[1], E. FEDELE[2], D. PUZZO[3], C. REBOSIO[2], W. GULISANO[3], A. PALMERI[3], D. PAES[1], L. WENNOGLE[4], Y. PENG[4,5], D. BERTRAND[6], J. PRICKAERTS[1];
[1]Maastricht Univ., Maastricht, Netherlands; [2]Univ. of Genoa, Genoa, Italy; [3]Univ. of Catania, Catania, Italy; [4]Intra-Cellular Therapies Inc, New York City, NY; [5]Rutgers Cancer Inst. of New Jersey, Rutgers Univ., New Brunswick, NJ; [6]HiQScreen Sàrl, Geneva, Switzerland

Abstract: α7 nicotinic acetylcholine receptors (α7nAChRs) are ligand-gated ion channels that have been implicated in modulating cognitive functions like episodic memory and attention. Hence, α7nAChR agonists/modulators have been investigated as potential drug candidates to improve cognition in Alzheimer's disease (AD) and schizophrenia, with the partial agonist EVP-6124 (Encenicline) recently terminated in Phase III clinical trials. Upon repeated activation the α7nAChR quickly desensitizes, possibly limiting the effects of (partial) agonists. In the current study, the cognition enhancing properties of low dose administration of selective α7nAChR antagonists were investigated in rats as low doses of methyllycaconitine (MLA) have been reported to improve cognition in animals. Memory acquisition and consolidation processes were assessed separately with the object recognition task (ORT). The compounds used for these studies were MLA and Compound 7i. Interestingly, it was found that low doses of either MLA or Compound 7i improved the acquisition, but not the consolidation processes of object recognition memory at a 24 h retention interval. Conversely, higher doses impaired the memory performance at a shorter 1 h retention interval. In addition, the same compounds were studied in a model of neuronal plasticity, long-term potentiation (LTP). In accordance to the behavioral studies, it was demonstrated that pre-tetanus low-dose administration of MLA or Compound 7i produced a longer lasting potentiation, whereas post-tetanus administration had no effect. Microdialysis studies showed that MLA administration substantially increased hippocampal glutamate efflux which has been found to be related to object memory processes. To further elucidate the mechanism by which these α7nAChR antagonists exerted this paradoxical effect,

electrophysiological studies in oocytes expressing human α7nAChRs were performed. These studies will be outlined and have led to novel a mechanistic model. The proposed model is an extension of the minimal allosteric model of ion channels in which low concentrations of α7nAChR antagonists re-sensitize the receptor channel by reversing the desensitized state to the resting state, which is again susceptible to agonist binding and subsequent channel opening. In summary, blocking α7nAChRs with low doses of selective antagonists improves specifically the memory acquisition process. While the main focus of the α7nAChR as a target for cognition enhancement has been on agonists and positive modulators, antagonism of these receptors at low doses might actually prove to be a valuable approach for cognition enhancement in AD or schizophrenia.

Disclosures: N.P. Van Goethem: None. **E. Fedele:** None. **D. Puzzo:** None. **C. Rebosio:** None. **W. gulisano:** None. **A. Palmeri:** None. **D. Paes:** None. **L. Wennogle:** None. **Y. Peng:** None. **D. Bertrand:** None. **J. Prickaerts:** None.

Poster

741. Cholinergic Modulation: Physiology and Behavior

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Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 741.23/JJJ1

Topic: H.01. Animal Cognition and Behavior

Title: Cognitive deficits in a rat model of Parkinson's disease are reversed by low dose acetylcholinesterase inhibition and serotonin 5-HT_{2A} inverse agonism

Authors: *D. T. HUBBARD, E. S. BURSTEIN, C. BELLIZZI, K. MCFARLAND; Biosci., ACADIA Pharmaceuticals, San Diego, CA

Abstract: Parkinson’s disease (PD) is primarily associated with motor impairment, yet non-motor symptoms, including behavioral and cognitive disturbances, are prevalent and greatly impact daily functioning and quality of life. Current treatments for PD dementia include administration of acetylcholinesterase inhibitors (AChEIs) which can be accompanied by undesirable side effects. Lowering the effective dose of AChEI could reduce the prevalence or severity of these side effects. The current work examined the potential of using low-doses of donepezil or rivastigmine in conjunction with a selective 5-HT_{2A} antagonist/inverse agonist called M100,907 to improve cognition in a rat model of PD. Sprague Dawley rats received bilateral sham or 6-hydroxydopamine (6-OHDA) lesions of the substantia nigra (SN)[1]. Following recovery, rats were administered M100,907, either donepezil or rivastigmine, or combinations of M100,907 with either donepezil or rivastigmine prior to assessment of novel object recognition (NOR) or working memory performance in the radial arm maze (RAM). 6-OHDA lesion

impaired the cognitive performance of rats in the NOR and RAM tests. Treatment with either rivastigmine or donepezil attenuated the cognitive deficits of 6-OHDA lesion rats, while M100,907 was ineffective. In addition, sub-effective doses of rivastigmine or donepezil combined with M100,907 reversed the deficits of lesioned rats in the NOR and RAM tasks. Pharmacokinetic studies suggest that the increased potency of either rivastigmine or donepezil cannot be explained by changes in their bioavailability as a consequence of co-administration with M100,907. This work suggests that rats with 6-OHDA lesion of the SN display cognitive deficits. Further, combined administration of low doses of AChEIs and selective 5-HT_{2A} inverse agonists, while ineffective alone, could be effective treatments of cognitive deficits associated with dopaminergic cell death, potentially with fewer side effects.

Disclosures: **D.T. Hubbard:** A. Employment/Salary (full or part-time): Employee of ACADIA Pharmaceuticals. **E.S. Burstein:** A. Employment/Salary (full or part-time): ACADIA Pharmaceuticals. **C. Bellizzi:** A. Employment/Salary (full or part-time): ACADIA Pharmaceuticals. **K. McFarland:** A. Employment/Salary (full or part-time): ACADIA Pharmaceuticals.

Poster

741. Cholinergic Modulation: Physiology and Behavior

Location: Halls B-H

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Program#/Poster#: 741.24/JJJ2

Topic: H.01. Animal Cognition and Behavior

Support: DL-NAT VIEP-BUAP

LAB NEURPHARM,ACHOLOGY 2016-L

Title: Effect of a new benzoazocine on learning, spatial memory, and oxidative stress on acetylcholinesterase activity in rats.

Authors: *I. LIMON PEREZ DE LEON[1], A. PATRICIO-MARTINEZ[1], N. MUÑOZ-MORENO[1], R. LOPEZ-GONZALEZ[2], J. TERAN-VAZQUEZ[2], B. LEON-CHAVEZ[3]; [1]Benemerita Univ. Autonoma De Puebla FCQ Lab. Neurofarma, Puebla, Mexico; [2]Ctr. de Quimica, BUAP, Puebla, Mexico; [3]Lab. de pruebas preclinicas, BUAP, Puebla,Pue, Mexico

Abstract: It have been reported that the Benzoazocines are structures azocines and that may have inhibitory activity on acetylcholinesterase (AChE). Drugs that enhance cholinergic activity through by reversible inhibition of the enzyme acetylcholinesterase and increase synaptic levels of acetylcholine is plausible that improves attention, learning and the memory process. If these

new benzoazocines produce these effects, it would be candidates to improve cognitive processes in neurodegenerative diseases such as Alzheimer's disease. Some acetylcholinesterase inhibitors such as galantamine reversibly inhibit AChE only up to 60%, increasing acetylcholine levels, resulting in an improvement in learning and memory processes. The aim of this study was to evaluate the effect of administration of a novel compound benzoazocines on learning and spatial memory. Also we investigated the effect on AChE activity in intact rats. Male Wistar rats were used. The animals were divided into control group (n = 7) and problem group (n = 7) and were administered subcutaneously [DMSO 0.5%] and 1.5 mg / kg of benzoazocines respectively, for 19 days. Spatial learning was assessed in the radial maze 10 days after administration. Memory was assessed on 21 day in the 8-arm radial maze. After the memory test, the animals were euthanized and extracted: frontal and temporal cortex, amygdala and hippocampus. Using spectrophotometer test the lipid peroxidation and nitrite determinations were made. The AChE activity was determined by the Ellman assay. The Benzoazocines (1.5 mg / kg) administration resulted in no change in the learning test. However there was an increase by 20% correct responses in the test of spatial memory compared to the control group. This dose caused no harmful effects on the system, with no induction of lipid peroxidation, or oxidative stress or nitrosative stress, compared to their respective control group. The administered dose (1.5mg / kg) produced an increase of 30% in the inhibition of AChE activity hippocampus and temporal cortex. There were not changes in amygdala and frontal cortex. The results provide the basis of study on that new structures are benzoazocines as compounds with anticholinesterase function and plausible effect on improving memory.

Disclosures: **I. Limon Perez De Leon:** A. Employment/Salary (full or part-time): Neuropharmachology Laboratory BUAP. **A. Patricio-Martinez:** None. **N. Muñoz-Moreno:** None. **R. Lopez-Gonzalez:** None. **J. Teran-Vazquez:** None. **B. Leon-Chavez:** None.

Poster

741. Cholinergic Modulation: Physiology and Behavior

Location: Halls B-H

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Program#/Poster#: 741.25/JJJ3

Topic: H.01. Animal Cognition and Behavior

Title: Does nicotine exposure potentiate the neurotoxic effects of prenatal alcohol-induced cognitive impairment?

Authors: ***M. A. MAJRASHI**, D. BHATTACHARYA, S. BHATTACHARYA, J. BLOEMER, M. BUABEID, M. ESCOBAR, P. DAS PINKY, V. SUPPIRAMANIAM, M.

DHANASEKARAN;
Harrison Sch. of Pharm. /auburn Univ., Auburn, AL

Abstract: Women (10-20%) have been found to consume large amount of alcohol (ethanol) during pregnancy. Prenatal alcohol exposure has been correlated to multitude of dose dependent effect on the structure and functions of the central nervous system in the humans and animals. During the pregnancy, in addition to alcohol consumption, smoking (nicotine exposure) is also observed. Thus, alcohol (ethanol) and smoking (nicotine) exposure during pregnancy can have substantial neurotoxic effects on the offspring. Therefore, in our study, we used a Sprague Dawley rat model exposed to alcohol (mixed with water) and nicotine (subcutaneous-mini osmotic pump) during gestation. We assessed the effects of alcohol and nicotine exposure on the behavioral and neurological changes. Y-maze was used to study the effect on cognitive impairment. Long-term potentiation (LTP) and expression of ILK & PSD-95 was studied to correlate the effects with cognition. Significant deficits in spatial memory task were observed in the alcohol only treated group as compared to the control. Interestingly, the offspring exposed to prenatal nicotine and alcohol showed significant improvement in the spatial task as compared to the alcohol treatment. However, the improvement in spatial learning deficit is not supported by the LTP in these animals. Alcohol and nicotine exposed animals showed significant deficit in LTP as compared to the control. There was an increase in the hippocampal PSD-95 expression and no change in ILK expression in the alcohol and nicotine treated group as compared to alcohol alone or the control. Thus, nicotine and alcohol exposure during pregnancy may have a mixed and detrimental effect in the central nervous system.

Disclosures: **M.A. Majrashi:** None. **D. Bhattacharya:** None. **S. Bhattacharya:** None. **J. Bloemer:** None. **M. Buabeid:** None. **M. Escobar:** None. **P. Das Pinky:** None. **V. Suppiramaniam:** None. **M. Dhanasekaran:** None.

Poster

741. Cholinergic Modulation: Physiology and Behavior

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Program#/Poster#: 741.26/JJJ4

Topic: H.01. Animal Cognition and Behavior

Support: BBSRC Grant BB/L001896/1

Title: The role of hippocampal to medial prefrontal cortex projections, and effects of acetylcholine manipulation in these regions, during an associative recognition memory task

Authors: *K. E. GILROY, P. J. BANKS, G. R. I. BARKER, E. C. WARBURTON, Z. I. BASHIR;
Physiology, Pharmacol. and Neurosci., Univ. of Bristol, Bristol, United Kingdom

Abstract: A series of experiments were conducted in a cross-maze using a behavioural design based on the Object-in-Place (OiP) test of associative recognition memory. An intact tripartite network between the hippocampus, perirhinal cortex and prefrontal cortex has previously been demonstrated to be critical for successful performance during the OiP task. Optogentic manipulations were used to determine the nature of the interactions between the hippocampus and medial prefrontal cortex at various stages of the task and pharmacological manipulation of acetylcholine was used to determine the possible role of neuroplasticity in these two regions. The results are discussed in relation to the tripartite network and the formation of associative recognition memory.

Disclosures: K.E. Gilroy: None. **P.J. Banks:** None. **G.R.I. Barker:** None. **E.C. Warburton:** None. **Z.I. Bashir:** None.

Poster

742. Modulation of Cognition and Behavior I

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Program#/Poster#: 742.01/JJJ5

Topic: H.01. Animal Cognition and Behavior

Support: FC-UNAM2016

Title: Electromagnetic forced stimulation alters differentially spatial learning processes depending on the time of the day.

Authors: *M. E. ARIAS-GARCÍA, D. BUSTAMANTE-VALDEZ, M. VÁZQUEZ-SUMANO, N. ORTEGA-VILLEGAS, P. DURAN;
LBAE, Facultad de Ciencias, Univ. Nacional Autonoma De México, Ciudad de México, Mexico

Abstract: Currently the exposure to electromagnetic waves is increasing due to the presence of household appliances related to the electric current and the emission of low-frequency waves, as well as radio stations, TV antennas, telecommunication signals, Wi-Fi, smartphones, etc. Damages to the health have been linked to them, however, transcranial electromagnetic stimulation has been used has therapy for epilepsy, depression, PTSD with positive results in humans, in animal models there is experimental evidence that at an particular intensity, magnetic stimulations normalize corticosterone levels in stressed animals, as well as when the organism is

exposed to non-ionizing radiation neurogénesis in the hippocampal dentate gyrus turns out to be favored. Also, there is experimental evidence about how magnetic stimulation can alter the learning and memory processes, in here, there is controversy, since some authors refer a positive meanwhile others found deleterial effects. Magnetic stimulation at 8mT, has been found to decrease consolidation in the reference memory using the Morris maze. The aim of this study was to determine if the forced exposure to electromagnetic waves of low frequency (0.2mT) in a particular moment of the day generates positive results in learning processes of learning and reference memory in adult rats. Sprague-Dawley adult male rats, were randomly divide in 6 groups (n=10), maintained under a 12/12 light cycle, water and food ad libitum, temp 22-24°C in polycarbonate cages. All groups were confined to a faradized chamber for 15 minutes every day for 3 weeks. Control (Co) was confined but not stimulated, meanwhile 2 groups were confined and stimulated (0.2mT) at ZT2 (means 2hrs after lights on), and 2 other groups were confined and stimulated at ZT14 (2hr after lights off). After 3 weeks all groups were tested using the Morris water maze, as follows ZT2 group was subdivide in ZT2/ZT3 and ZT"/ZT15, meaning animals were tested at ZT3 or ZT15. ZT14 groups were under similar treatment, ZT14/ZT3 andZT14/ZT15. Controls were tested at ZT3 and ZT15. Our results shows that total acquisition latency improved in all stimulated groups, those were faster learners as well. Consolidation latencies were improved in ZT2/ZT3 and ZT14/ZT3, but worsened in ZT2/ZT15 and ZT14/ZT15 groups. For reference memory Probe immediately afterwards, we found no significant differences, but after 8 days memory was poorer than control. We conclude that although 0.2mT appears to be positive for learning, there is a temporal component may be linked to the circadian attention processes that affect the memory consolidation and long term retrival.

Disclosures: **M.E. Arias-García:** None. **D. Bustamante-Valdez:** None. **M. Vázquez-Sumano:** None. **N. Ortega-Villegas:** None. **P. Duran:** None.

Poster

742. Modulation of Cognition and Behavior I

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Program#/Poster#: 742.02/JJJ6

Topic: H.01. Animal Cognition and Behavior

Support: DCBS UAM

CONACyT Fellow Ship JHR

Title: Chronic cadmium administration alter learning and memoryprocess in male Wistar rat

Authors: *M. ARTEAGA-SILVA[1], R. VILLANUEVA-REYES[2], S. MONTES[5], C. ESPINOSA GARCÍA[6], A. ROMERO[2], N. A. ESTRADA-CRUZ[3], J. HERNÁNDEZ-RODRÍGUEZ[4], H. BONILLA-JAIME[4], P. G. DAMIAN-MATZUMURA[4], R. M. VIGUERAS VILLASEÑOR[7], P. DURAN[8];
[2]Biología, [3]Ciencias de la Salud, [4]Biología de la Reproducción, [1]Univ. Autónoma Metropolitana-Iztapalapa, Méxcio, Mexico; [5]Neuroquímica, Inst. de Neurología y Neurocirugía, México, Mexico; [6]Univ. of Emory, Atlanta, GA; [7]Inst. Nacional de Pediatría, Mexico, Mexico; [8]Facultad Ciencias, UNAM, México, Mexico

Abstract: The cadmium (Cd) is a highly toxic heavy metal and have been determined that Cd affects some organs such as the liver and kidney. Apparently, the brain is also affected and interferes with the cognition function. However, for the time there are few studies dealing with the effects of the Cd over the learning and the spatial memory and his correlation with the concentration of the Cd in the hippocampus, and the blood serum. To carry out this study, pregnant female rats, where used and monitored until parturition. The eight male pups were assigned by one mother. They were kept in a light-dark 12:00 to 12:00, the pups were injected ip with cadmium chloride at doses of 1 mg/kg /600 µl injection and control group were injected with saline. The injections were daily up to 56 days of life. The learning test was performed at 120 days of age, per six consecutive days, and the spatial memory in the 7th day using the aquatic maze of Morris (AMM). Finally after the last register (day 7), the subjects (Ss) were sacrificed by decapitation with anesthesia for obtaining serum and hippocampus, it was also determined the concentrations of Cd by atomic absorbance spectrophotometry. The results on the learning and especial memory in the AMM showed that the treated group with Cd his learning decreased significantly compared with the control group. On day seven, the Ss of control group showed a persistent searching on the platform, while Ss of control group with Cd weren´t persistent in the searching of the platform and Cd concentration in hippocampus and serum blood were increased in the experimental group. These data suggest that Cd chronic administration was accumulated in hippocampus, and may provoke alterations in this structure altering the learning and spatial memory processes.

Disclosures: M. Arteaga-Silva: None. **R. Villanueva-Reyes:** None. **S. Montes:** None. **C. Espinosa García:** None. **A. Romero:** None. **N.A. Estrada-Cruz:** None. **J. Hernández-Rodríguez:** None. **H. Bonilla-Jaime:** None. **P.G. Damian-Matzumura:** None. **R.M. Vigueras Villaseñor:** None. **P. Duran:** None.

Poster

742. Modulation of Cognition and Behavior I

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Program#/Poster#: 742.03/JJJ7

Topic: H.01. Animal Cognition and Behavior

Support: 2015BCFC-UNAM

Title: Acute E14 Cadmium administration alters water intake circadian rhythm, visual perception, anxiety and stress responses in the rat

Authors: J. S. GONZALEZ-RUANO, E. MORENO-SÁENZ, M. E. ARIAS-GARCÍA, D. J. BUSTAMANTE-VALDEZ, *P. DURAN;
Facultad De Ciencias, UNAM, Ciudad de Mexico, Mexico

Abstract: Cadmium (Cd) is a heavy metal profusely distributed in the environment as a pollute from human industrial activities It is known to be potentially teratogenic even lethal during the development. Its teratogenic effects are mainly found in neural tube formation, renal, skeletal, heart and immune system. In mice there is evidence that a single dose of Cd administrate during the retinal ganglion cells (RGC) proliferative critical period induce the dead of RGC in the offspring. Intrinsically photosensitive Retinal Ganglion Cells (ipRGCs) are known to participate in the circadian synchronization, in rats the proliferative critical period of those cells has been found at E14. The aim of this study was to determine if the acute administration of Cd at E14 produce postnatal alterations in the water intake circadian rhythm. We followed the early postnatal development markers, and performed a visual perception task at a juvenile postnatal age. We found not differences in synchronization when compared with controls, but an increase in the rhythm amplitude, and delay of 2 or 3 days in development markers, as well we found the acute Cd administration had an effect in the anxiety and stress behavioral markers in the open field of visual cliff task. Those results suggest the acute E14 Cd administration is enough to produce behavioral and physiological effects on those structures developing during that particular period.

Disclosures: J.S. Gonzalez-Ruano: None. **E. Moreno-Sáenz:** None. **M.E. Arias-García:** None. **D.J. Bustamante-Valdez:** None. **P. Duran:** None.

Poster

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Topic: H.01. Animal Cognition and Behavior

Support: FC-UNAM 2016

Title: Prenatal nicotine exposure alters stress- anxiety circadian rhythm in female mice

Authors: *M. FUENTES-CANO, L. G. MENDOZA-MORENO, P. DURAN;
Facultad De Ciencias, UNAM, Mexico City, Mexico

Abstract: Smoking and nicotine use during pregnancy has been associated with several adverse effects of growth and perinatal development. Abortion, low weight at birth, delayed postnatal development and changes in cognitive performance are among those detrimental effects. Acetylcholine is one of the most important signalling brain developmental processes which can be interfered by nicotine interaction with nicotinic cholinergic receptors (nAChr). There is also evidence that nicotine itself can disturb sex hormones patterns in both female and male adults, as well as in offspring exposed to nicotine during pregnancy and lactation. Nicotine consumption may be affecting critical periods during which certain neural circuits are formed, resulting in modifications in behavioural sexual dimorphism. The aim of the present study was to evaluate the effect of perinatal nicotine administration on the daily rhythm of stress and anxiety-like response in the juvenile mice. BALB/c female mice were given perinatal nicotine diluted in water (6 mg/kg/day -Slotkin, 1995-). After offspring delivery, litters were weighted and developmental markers were taken in order to assess the model (Ajarem, 1998). Only female offspring between 30-40 days old was used in the behavioural tests. The marble burying task was used as a model to measure stress and anxiety-like behaviour, during six temporal windows for three consecutive days. Ten nicotine-exposed females and ten control females were used in each temporal window. Mice were held on 12:12 LD conditions, with access to water and food *ad libitum*. Nicotine offspring showed a 2 to 3 days delay regarding developmental markers such as whiskers, eye opening, mobility, etc. After the behavioural test results suggest that control female mice exhibit a bimodal stress and anxiety response daily rhythm, with the highest levels peaking at the light phase, and a low response level between them, in the middle of the light phase. On the other hand, perinatal exposure to nicotine appears to produce modifications to this daily rhythm, elevating stress response levels during the middle of the light phase.

Disclosures: **M. Fuentes-Cano:** None. **L.G. Mendoza-Moreno:** None. **P. Duran:** None.

Poster

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Memolife LABEX

Title: Extradimensional attentional set-shifting related changes in prefrontal and striatal neuronal activity.

Authors: V. OBERTO, H. GAO, M. ZUGARO, *S. I. WIENER;
Col. De France CIRB, Paris, France

Abstract: Cortical connectivity with the striatum (Str) involves parallel yet overlapping anatomical 'loops'. Prefrontal (Pfc)-Str loops are implicated in goal directed learning processes and flexible attentional set-shifting. Here we examine changes in neural activity during set-shifting via simultaneous recordings of multiple single neuronal activity and local field potentials in 7 rats from areas including dorsomedial and ventral Str and cingulate, prelimbic and infralimbic areas of Pfc. The rats switched between two different reward-response contingencies in an automated T-maze with return arms. First they performed a brightness discrimination task (*VC)* where the rewarded arm was cued by video displays behind the reward arms, varying pseudorandomly between trials. Upon reaching criterion they were challenged with a spatial orientation task (*T*, reward always on the animal's non-preferred side: left or right) while (inconsequential) visual cues were still present. Extradimensional task switches continued whenever criterion performance was reached. Note that the only cue indicating the current rule was the presence or absence of the liquid reward. Here we focus analyses on sessions where rats successively reached criterion in VC (called 'VC1'), then T, and then VC again (called 'VC2'). We analysed 469 Str and 231 Pfc neurons with a bootstrap Monte Carlo method to detect increases or decreases in firing rate among the criterion performance trial epochs: VC1, T and VC2. Activity in these three conditions was compared separately for each of 15 bins along the central arm of the maze. The sampling of subregions of Pfc or of Str showed no differences for the three epochs. About 20% of the neurons in both Str and Pfc showed location-specific activity level increases or decreases in pair-wise comparisons among VC1, T or VC2 epochs. Surprisingly, both in Str and Pfc the highest incidences of these activity shifts were for VC1 vs. VC2 comparisons. In Str, the numbers of cells with activity shifts were: 37 for VC1 vs T; 29 for T vs VC2 and 51 for VC1 vs VC2. In Pfc the numbers of responsive cells were: 11 for VC1 vs T; 18 for T vs VC2 and 22 for VC1 vs VC2. The high incidence of VC1 vs VC2 activity shifts indicates that the firing rates of the neurons do not simply consistently code for a particular task but rather might be sensitive to other factors such as the history of recent behavioral challenges, possibly impacting on the level of motivation or fatigue of the animal. Furthermore the distribution of incidences of shifts for the three comparisons appears be different in Pfc vs Str. If confirmed this could reflect differences in executive and decisional processing in the two structures.

Disclosures: **V. Oberto:** None. **H. Gao:** None. **M. Zugaro:** None. **S.I. Wiener:** None.

Poster

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Topic: H.01. Animal Cognition and Behavior

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French Agence National pour la Recherche [Grant ANR-10-BLAN-02 (Neurobot)]

Title: Locus coeruleus task-related activity in an attentional-set shift task.

Authors: L. XIANG[1], A. HAREL[1], S. I. WIENER[1], *S. J. SARA[2];
[1]CIRB, [2]Collège de France, Paris, France

Abstract: The noradrenergic nucleus locus coeruleus (LC) plays a role in cognitive function, controlling vigilance states, orienting attention, modulating responses in sensory pathways and promoting long term memory consolidation. Recent fMRI studies in humans lend support to the hypothesis derived from rodent studies, that LC activation promotes rapid shifts in cortical attentional networks in response to a change in environmental contingencies. To further examine this hypothesis we designed a set-shift task in an automated T maze with video displays behind the reward arms and liquid rewards. Rats initially were required to learn a brightness discrimination task. Each trial was initiated by the rat when it left the start area and crossed a photocell that turned on the discriminanda. After criterion performance of 8 successive rewarded trials was reached, a spatial response-reward contingency was instated with brightness cues still present in the same pseudorandom sequence - the rat had to learn to ignore them and acquire the spatial rule for rewards. Well-trained rats underwent successive task shifts within a single session. The aim here was to determine the relevant task-related stimuli that elicited a response in the LC. After reaching criterion on the brightness discrimination, rats were anesthetized and implanted with a moveable microelectrode in LC, a tetrode in hippocampus and in the prelimbic area of prefrontal cortex. After recovery, rats were retrained to criterion on the brightness task, and then the response-reward contingency was switched to a non-preferred side turning task in the same session. Results indicate that LC neurons are engaged as the rat initiates the trial, with peak firing 500-200 ms *before* the onset of the visual cue. This activation occurred across all trials and contingency conditions and is reminiscent of previous reports that LC neurons respond preferentially to a preparatory stimulus in a formal conditioning protocol. However in this case, the preparatory stimulus would be internally generated. Other task-related LC responses observed consistently through different recording sessions include phasic activation just before crossing the reward arm photocell and almost total inhibition at reward consumption sites

(inhibition both in rewarded and non-rewarded trials). After this inhibition, LC cells were again phasically activated in the return arm, from 1 s before until crossing a photocell triggering the extinction of the visual cue, which signaled the end of the trial. These task-related responses of LC neurons will instruct future experiments using optogenetically-elicited gain or loss of LC function to study its role in cognition.

Disclosures: **L. Xiang:** None. **A. Harel:** None. **S.I. Wiener:** None. **S.J. Sara:** None.

Poster

742. Modulation of Cognition and Behavior I

Location: Halls B-H

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Program#/Poster#: 742.07/JJJ11

Topic: B.07. Synaptic Transmission

Support: NIH Grant R01DK106188

Brain & Behavior Research Foundation N018940

NIH Grant T32DA007268

Title: Effects of insulin on excitatory transmission in the nucleus accumbens of non-obese and obese rats

Authors: ***M. F. OGINSKY**, C. R. FERRARIO;
Univ. of Michigan, Ann Arbor, MI

Abstract: Excitatory transmission in the nucleus accumbens (NAc) mediates motivation for food. Infusion of insulin in the NAc increases motivation for food, but the mechanisms underlying this effect are poorly understood. Insulin decreases excitatory transmission in the cortex and ventral tegmental area and increases dopamine release in the NAc. Here, we determined the effects of insulin on excitatory transmission in the NAc core of non-obese and obese adult rats. Whole cell patch clamp recordings of evoked AMPAR mediated excitatory postsynaptic currents (eEPSCs) were made in NAc medium spiny neurons from rats fed lab chow (Lab Diet 5001) or 60% high-fat diet (Open Source Diets D12492; 8 wks). Bath application of insulin increased eEPSC amplitude at low concentrations (30 nM), but reduced eEPSC amplitude at higher concentrations (50, 100 or 500 nM). Because insulin has a high affinity for the insulin receptor (IR) and lower affinity for the insulin-like growth factor-1 receptor (IGF-1R) we hypothesized that increases in eEPSC amplitude at 30 nM were due to activation of IRs, while decreases at higher concentrations were due to activation of IGF-1Rs. To test this, we included the insulin receptor inhibitor, HNMPA (300 μM), in the recording pipette

or the IGF-1R antagonist, picropodophyllotoxin (PPP; 500 nM), in the bath before applying insulin. Consistent with our hypothesis, HNMPA blocked the ability of 30 nM insulin to increase eEPSC amplitude. In addition, when 100 nM insulin was applied in the presence of PPP, there was an *increase* in eEPSC amplitude. Together these data show that activation of IRs and IGF-1Rs produce opposing effects on excitatory transmission, with IR activation increasing and IGF-1R activation decreasing excitatory transmission. When recordings were made from obese rats, increases in excitatory transmission after 30 nM insulin were completely absent, while decreases after 100 nM insulin were comparable to those seen in chow fed controls. These data suggest that IR-mediated effects may be reduced after high-fat diet consumption, while IGF-1R-mediated effects remain intact. In addition, data from high-fat fed rats suggest that peripheral alterations induced by consumption of high-fat diet may significantly impact the ability of insulin to modulate neuronal function. In sum, our data show that in non-obese rats insulin bi-directionally influences excitatory transmission via dissociable actions on IRs and IGF-1Rs, and suggest that consumption of high-fat food selectively blocks IR-mediated increases in NAc excitatory transmission. These data shed light on potential mechanisms by which insulin may regulate motivational processes.

Disclosures: **M.F. Oginsky:** None. **C.R. Ferrario:** None.

Poster

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Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

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Topic: B.07. Synaptic Transmission

Support: Beckman Young Investigator Award

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Brain and Behavior Research Foundation – NARSAD Young Investigator Grant, P&S Fund Investigator

Rita Allen Foundation Scholar Award

Title: Dissecting the neuromodulatory landscape of the PVN

Authors: *L. XIAO[1], J. NASENBENY[1], M. PRIEST[1], T. LU[2], Y. KOZOROVITSKIY[1]; [1]Dept. of Neurobio., [2]Dept. of Communication Sci. and Disorders, Northwestern Univ., Evanston, IL

Abstract: Oxytocin is a hormone and neuromodulator that regulates central nervous system to control social behavior. Oxytocin neurons are primarily concentrated in two areas of the hypothalamus: paraventricular nucleus (PVN) and supraoptic nucleus (SON). Oxytocin neurons in the PVN, but not the SON, are responsible for the majority of central oxytocin projections. However, in addition to oxytocinergic neurons, the PVN contains many distinct neurotransmitter-expressing populations (*e.g.*, vasopressin, corticotripin-releasing factor, *etc*). Here, relying on several Cre recombinase-expressing mouse lines, as well as immunohistochemical and *in situ* hybridization techniques, we profile the neuromodulatory populations of PVN, together with their fast neurotransmitter content (*e.g.*, GABA), and begin to characterize the functional impact of neural activity with in specific PVN projections.

Disclosures: L. Xiao: None. **J. Nasenbeny:** None. **M. Priest:** None. **T. Lu:** None. **Y. Kozorovitskiy:** None.

Poster

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Topic: B.07. Synaptic Transmission

Support: NIH Grant R01MH099054

Title: Chronic SSRI treatment promotes inhibitory serotonergic signaling in rat prefrontal cortex

Authors: *E. K. STEPHENS[1], D. AVESAR[1], T. P. TODD[3], D. J. BUCCI[3], S. GERBER[2], A. T. GULLEDGE[1];
[1]Physiol. & Neurobio., [2]Genesis and Biochem., Geisel Sch. of Med. At Dartmouth, Lebanon, NH; [3]Psychological and Brain Sci., Dartmouth Col., Hanover, NH

Abstract: The selective serotonin reuptake inhibitor (SSRI) fluoxetine is one of the most widely prescribed drugs for the treatment of depression and anxiety. SSRIs directly and immediately increase the amount of serotonin (5-HT) in the brain by blocking 5-HT transporters, yet little is known about how elevated 5-HT levels contribute to the delayed therapeutic efficacy of SSRIs, which develops slowly over several weeks. Here we demonstrate that chronic treatment with fluoxetine alters the physiological impact of 5-HT on pyramidal neurons in the rat prefrontal cortex (PFC). Unlike in the mouse PFC, where serotonergic responses are strictly segregated among callosal/commissural projection (COM) and corticopontine (CPn) neurons, serotonergic signaling in the rat PFC is more variable among projection neuron subtypes. Similar to the mouse, COM neurons in rat (94%; n = 49/52) exhibited excitatory responses to 5-HT via

activation of 5-HT_{2A} (2A) receptors, either alone (n = 32/52), or as part of biphasic responses involving an initial 5-HT_{1A} (1A) receptor-mediated inhibition (n = 17/52). A few rat COM neurons (6%; n = 3/52) exhibited purely inhibitory responses to 5-HT. More surprisingly, whereas almost all CPn neurons in the mouse PFC are inhibited by 5-HT, most CPn neurons in the rat PFC exhibited 2A-dependent excitatory (68%; n = 40/59) or biphasic (3%; n = 2/59) responses. Only 19% (n = 11/59) of rat CPn neurons exhibited pure 1A-dependent inhibition. The proportions of CPn neurons excited by 5-HT did not differ with sex or across animal strains, including Long Evans, Kyoto/Wistar, and Sprague Dawley. Additionally, manipulations such as enriched environments, or housing in a different facility, did not affect 5-HT responsivity in rat CPn neurons. However, chronic treatment with the SSRI fluoxetine (7 mg/kg/day) for 21 days via a subcutaneous osmotic pump, shifted serotonergic responsiveness in rat CPn neurons, such that 5-HT inhibited the majority (56%; n = 44/79) of neurons, while only 38% (n = 30/79) of CPn neurons exhibited excitatory (n = 27) or biphasic (n = 3) responses ($p < 0.01$; Fisher's Exact Test). The impact of chronic fluoxetine was selective for CPn neurons, as 5-HT responses in COM neurons remained qualitatively unchanged following fluoxetine-treatment, with 61% (n = 36/59) excited, 34% (n = 20/59) biphasic, and 3% (n = 2/59) inhibited by 5-HT. Serum analyses confirmed that fluoxetine-treated animals had 9.4 ng/mL fluoxetine, a level significantly above that detected in vehicle-treated animals (1.4 ng/mL; $p < 0.05$; Student's t-test). These data reveal a novel form of modulatory plasticity in rat PFC that may contribute to the delayed therapeutic action of SSRIs.

Disclosures: **E.K. Stephens:** None. **D. Avesar:** None. **T.P. Todd:** None. **D.J. Bucci:** None. **S. Gerber:** None. **A.T. Gulledge:** None.

Poster

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Program#/Poster#: 742.10/JJJ14

Topic: B.07. Synaptic Transmission

Support: NIH R01 MH101178

Title: Corticotrophin releasing factor dose-dependently modulates excitatory synaptic transmission in the noradrenergic nucleus locus coeruleus

Authors: *B. D. WATERHOUSE[1], D. J. CHANDLER[2];
[2]Neurobio. and Anat., [1]Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: The noradrenergic nucleus locus coeruleus (LC) is a critical node in the stress response and receives afferent input from a number of corticotrophin releasing factor (CRF) containing structures. Several *in vivo* and *in vitro* studies have shown that CRF robustly increases the firing rate of LC neurons in a dose-dependent manner. While it is known that these increases are dependent on CRF receptor subtype 1 and mediated by effects of the protein kinase A/cAMP intracellular signaling cascades on potassium conductance, the impact of CRF on synaptic transmission within LC has not been clarified. In the present study, we used whole-cell patch clamp electrophysiology in horizontal brainstem slices to assess how varying concentrations of bath-applied CRF affected AMPA-receptor dependent spontaneous EPSCs. Compared to vehicle, 10nM and 25nM CRF had no significant effects on any sEPSC parameters. 50nM CRF, however, significantly increased sEPSC amplitude, width, and charge transfer, while amplitude and charge transfer were significantly decreased by 200nM CRF. While 100nM CRF did not affect the mean change as compared to vehicle, it did significantly increase the variability of the response, such that approximately half of the neurons exposed to this dose displayed increased sEPSC amplitudes, while the other half showed decreased amplitudes. These observations suggest that stress may differentially affect ongoing excitatory synaptic transmission in LC depending on how much CRF is released from presynaptic terminals. Specifically, mild or moderate stressors may potentiate excitatory synaptic transmission, thereby increasing phasic LC responses to salient stimuli, whereas intense stressors might dampen these responses. Combined with the well-documented effects of CRF on membrane properties and spontaneous LC discharge, these observations may help explain how stress and CRF release are able to simultaneously increase tonic LC discharge and decrease sensory-driven phasic discharges. These findings have implications for how stress affects the fidelity of signal transmission and information flow through LC and how norepinephrine release in cognitive, sensory, and motor circuitries and ensuing behaviors might be impacted.

Disclosures: **B.D. Waterhouse:** None. **D.J. Chandler:** None.

Poster

742. Modulation of Cognition and Behavior I

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Topic: B.07. Synaptic Transmission

Support: NIH Grants R01 DA035217

NIH Grants R56 MH101146

Title: Pharmacological inhibition of diacylglycerol lipase α, but not diacylglycerol lipase β, blocks endocannabinoid-mediated retrograde synaptic depression

Authors: *X. LIU[1], J. TONG[1], C. VICKSTROM[1], D. OGASAWARA[2], H. DENG[3], M. STELT[3], B. F. CRAVATT[2], Q.-S. LIU[1];
[1]Med. Col. of Wisconsin, Milwaukee, WI; [2]the Scripps Res. Inst., La Jolla, CA; [3]Leiden Univ., CC Leiden, Netherlands

Abstract: The endocannabinoid 2-arachidonoylglycerol (2-AG) mediates multiple forms of retrograde synaptic depression including depolarization-induced suppression of excitation (DSE), inhibition (DSI) and synaptically evoked suppression of excitation (SSE) induced by activation of group I metabotropic glutamate receptors (mGluRs). Diacylglycerol lipases (DAGLα and DAGLβ) are biosynthetic enzymes for 2-AG. However, previously known DAGL inhibitors cannot distinguish between DAGLα and DAGLβ, and conflicting results have been reported regarding whether DAGL inhibitors block DSE and DSI. Here, using the recently-developed selective DAGLβ inhibitor KT109 and dual DAGLα/β inhibitors DO34 and DH376, we investigated the effects of acute, selective pharmacological inhibition of DAGLα and DAGLβ on DSE, DSI and SSE in Purkinje cells in mouse cerebellar slices. We report that dual DAGLα/β inhibitors DO34 and DH376 blocked DSE, DSI and SSE in Purkinje cells of the cerebellum, whereas selective DAGLβ inhibitor KT109 did not have significant effects. This pharmacological profile could also be extended to DSI in CA1 pyramidal neurons in the hippocampus and medium spiny neurons in the dorsal striatum. Taken together, these results provide pharmacological evidence that 2-AG synthesized by DAGLα, but not DAGLβ, is required for endocannabinoid-mediated retrograde synaptic depression.

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Poster

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Program#/Poster#: 742.12/JJJ16

Topic: B.07. Synaptic Transmission

Title: Shifting the mineralocorticoid and glucocorticoid receptor balance affects early life stress related effects on hippocampal glutamatergic transmission

Authors: *H. KARST[1], M. LOI[2], S. KANATSOU[2], H. J. KRUGERS[2], M. JOELS[1]; [1]Univ. Med. Ctr. Utrecht, Utrecht, Netherlands; [2]SILS-Center for Neurosci., Univ. of Amsterdam, Amsterdam, Netherlands

Abstract: Stressful events early in life can increase the risk to develop psychiatric disorders. In several models for early life stress (ELS) in rodents, it was shown that hippocampal learning tasks during adulthood were impaired. In these ELS animals also long term potentiation (LTP) in slices of the hippocampus was affected. Recent studies indicate that the functionality of the corticosteroid receptors may be important for the risk to develop ELS related disorders. Here we studied which of the two corticosteroid receptors, the glucocorticoid (GR) or mineralocorticoid receptor (MR), could be responsible for changes in the glutamatergic transmission, induced by ELS in rodents. In 3 months' old rats which were maternally deprived for 24 hours at postnatal day (PND) 3, we recorded in vitro miniature excitatory postsynaptic currents (mEPSCs) in hippocampal CA1 neurons. ELS dramatically decreased the frequency of the mEPSCs , by almost 70% . The amplitude of the mEPSCs was not affected. To study the role of the GRs, we treated rats with Mifepristone for three days at PND 26-28. The treatment with this GR antagonist completely rescued the effect of ELS, measured in the adult rats. To study the role of the MRs after ELS, we used mice with an overexpression of the MR (MRtg). In these experiments we induced ELS in MRtg and control mice with the limited bedding/nesting model. With this ELS model we also detected a reduction in the mEPSC frequency in the hippocampus (dentate gyrus) in control mice. In the MRtg mice, however, ELS did not affect the mEPSC frequency anymore. In none of the tested animals, the amplitude of the mEPSCs was affected. We conclude that when the GRs are suppressed or the MRs are brought to overexpression, the effect of ELS on hippocampal glutamatergic transmission is reduced. These results indicate that the balance between GR and MR may predict vulnerability to stress.

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Poster

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Topic: B.07. Synaptic Transmission

Support: NIH Grant DA019112

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Title: Uncovering the effects of agonism at postsynaptic α_{2A}-adrenergic receptors on neuronal activity within the bed nucleus of the stria terminalis

Authors: *N. A. HARRIS[1,2,3,4,5], A. T. ISAAC[2], S. A. FLAVIN[3], D. G. WINDER[1,2,3,4,5];
[2]Mol. Physiol. and Biophysics, [3]Vanderbilt Brain Inst., [4]Neurosci. Program in Substance Abuse, [5]Kennedy Ctr., [1]Vanderbilt Univ., Nashville, TN

Abstract: Stress is a major risk factor for relapse to drug-seeking behavior. The bed nucleus of the stria terminalis (BNST) receives an extensive noradrenergic input and has been shown to be critical in stress-induced reinstatement of drug-seeking behavior. In rodents, systemic or intra-BNST treatment with α_2-adrenergic receptor agonists decreases stress-induced reinstatement behaviors. Clinical studies report that guanfacine, an α_{2A}-AR agonist, curbs cravings for a variety of substances of abuse. We have previously shown that α_{2A}-ARs are heavily expressed in the BNST, and that noradrenergic signaling in the region is dramatically altered in α_{2A}-AR knockout mice. Data suggests that competing inhibitory and excitatory actions of this drug in the BNST could reflect carefully coordinated control of BNST activity. Our lab has shown inhibitory effects of guanfacine in the BNST on electrically evoked EPSCs and optically evoked EPSCs derived from parabrachial nucleus (PBN) afferents, consistent with canonical presynaptic G_i signaling. In contrast, in a Thy1-COP4 transgenic mouse line, bath application of guanfacine enhances optically evoked field potentials. In addition, systemic guanfacine is known to initiate c-fos expression in BNST neurons. These findings led us to pursue mechanistic studies of this effect. Using RNAscope *in situ* hybridization, we show that systemic guanfacine leads to expression of cfos transcripts in 90% of cells that express *adra2a* (α_{2A}-AR) mRNA, as compared to 10% of cells after saline. Interestingly, ~50% of cells that express cfos do not co-express *adra2a* mRNA, suggesting potential involvement of an underlying BNST microcircuit in this effect. We hypothesized that this novel excitatory effect occurs via activation of postsynaptic α_{2A}-ARs leading to decreased cAMP-dependent opening of hyperpolarization-activated cyclic nucleotide-gated nonselective cation (HCN) channels. This hypothesis is based on our observations that ~80% in cfos+ cells after systemic guanfacine in a cfos-eGFP mouse line exhibited a hyperpolarization-dependent sag consistent with Ih, as well as enhanced excitatory field potential responses in Thy1-COP4 BNST slices after HCN inhibition by ZD7288. However, RNAscope experiments show little co-localization of *adra2a* and *hcn1* mRNA, suggesting either *hcn2* involvement or independent mechanisms of activity enhancement. In addition, whole cell recordings show that guanfacine preincubation does not affect the amplitude of I_h in cfos+ BNST neurons after systemic guanfacine. Future studies will test the involvement of HCN2 channels and other candidates in guanfacine enhancement of BNST activity.

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Poster

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Topic: B.07. Synaptic Transmission

Support: NIH MH060605

NIH NS083319

Title: Differential modulation of synapses by neuropeptides with convergent actions on intrinsic excitability

Authors: *X. LI, D. BUCHER, F. NADIM;
Dept Biol. Sci., Rutgers/Njit, Newark, NJ

Abstract: Neuronal excitability and synaptic function depend on neuromodulators, and different modulators shape neural circuit activity into different patterns. However, circuits are likely under control of more than one modulator at all times. Therefore, understanding co-modulation is important for the study of circuit dynamics. Modulators can converge onto the same subcellular targets in a neuron, or affect different targets. In the case of true convergence, i.e. different modulator receptors sharing the same signaling pathway and subcellular target, the effects of two modulators should be simply additive until they occlude each other. In all other cases, interactions between two modulators can be complex and highly nonlinear. In the pyloric circuit of the crab stomatogastric ganglion (STG), several neuropeptides are converging onto the same voltage-gated current, occluding each other's effects. Different effects on circuit activity are attributed to the fact that each circuit neuron only responds to a subset of these peptides, i.e. each peptide activates a different subset of neurons. In addition, concentration-dependence and magnitude of the effect can be different between two neuropeptides. More recently, it has been shown that some peptides can also affect synapses. If both synaptic partners express receptors to the same peptide, the effects can potentially be mediated through both presynaptic and postsynaptic mechanisms. We studied the effect of proctolin and crustacean cardioactive peptide (CCAP) on the synaptic connections between the pyloric dilator (PD) and lateral pyloric (LP) neurons in the STG. Both PD and LP express receptors to proctolin, but only LP expresses receptors to CCAP. The effects of proctolin and CCAP on the voltage-gated current in LP are consistent with convergence and occlusion. However, the effects on synapses are more complex. PD and LP make reciprocal graded inhibitory connections, and we show that both synapses are strengthened by each neuromodulator, albeit in different fashion. At the PD to LP synapse, CCAP enhances the synaptic current amplitude while proctolin modulates its dependence on presynaptic voltage. At the LP to PD synapse, either modulator increases the amplitude of the synaptic current and changes dependence on presynaptic voltage. At both synapses, sequential

application shows that CCAP completely occludes the effect of proctolin. In contrast, CCAP application additionally increases synaptic current after application of proctolin. These results suggest that co-modulation of synaptic effects is more complex than simple convergence, with potential consequences for circuit modulation.

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Poster

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Topic: B.07. Synaptic Transmission

Support: Università Cattolica intramural funds Linea D.1-2014 and 2015

Title: High-fat diet causes hippocampal resistance to leptin-driven modulation of synaptic transmission

Authors: *M. MAINARDI, M. SPINELLI, F. SCALA, S. FUSCO, A. MATTERA, M. D'ASCENZO, C. GRASSI;
Inst. of Human Physiol., Catholic Univ. Sch. of Med., Rome, Italy

Abstract: Leptin plays an essential role in metabolic homeostasis, by signaling to the brain the level of long-term energy stores (i.e., white adipose tissue depots). Strikingly, abundant expression of ObRb - the functional isoform of the leptin receptor - can be detected also in the hippocampus, a structure not primarily involved in metabolic homeostasis, but central to learning and memory processes. Indeed, leptin has been shown to modulate hippocampal synaptic transmission and plasticity. Moreover, at the behavioral level, leptin is able to affect performance in hippocampus-dependent tasks. Taken together, these effects have led to postulating a neurotrophin-like role for leptin.
Since it is becoming increasingly clear that altered diet composition affects brain functioning, we asked whether a high-fat diet since weaning can lead in adulthood to altered response to the action of leptin on the hippocampus.
We first performed patch-clamp recordings on brain slices comprising the CA3 to CA1 circuit obtained from adult (age 3-4 months) mice fed a high-fat diet (HFD group) or a standard diet (SD group). We found that, in SD mice, bath application of leptin (50 nM) increased the frequency of spontaneous excitatory postsynaptic currents (sEPSCs), and that this effect was lost in HFD mice (SD = 144.25 ± 21.45%, HFD 98.44 ± 4.52% of baseline value; ANOVA-1, $p = 0.003$). Analysis of glutamatergic, AMPA receptor-mediated evoked currents revealed that leptin

increased amplitude in SD mice, whereas no significant difference was observed in HFD mice (SD = 166.11 ± 13.03%, HFD = 102.81 ± 9.85% of baseline value, ANOVA-1, $p < 0.001$). Moreover, a similar effect was observed on paired-pulse facilitation, which we evaluated over a wide range of inter-stimulus intervals: 200 ms (SD = 117.20 ± 6.58%, HFD = 104.37 ± 8.15%), 100 ms (SD = 114.19 ± 5.54%, HFD = 100.56 ± 4.42%), 50 ms (SD = 131.47 ± 9.46%, HFD = 99.88 ± 8.59%), 20 ms (SD = 136.10 ± 11.64%, HFD = 101.03 ± 7.19%; % of baseline values, ANOVA-3, $p < 0.001$).
In search of a molecular correlate of these functional phenomena, we checked the integrity of the STAT3 cascade, a key transducer of leptin action on neurons. In line with electrophysiological data, intraperitoneal injection of leptin (3 mg/kg) caused strong phosphorylation of STAT3 in SD mice, a response that was significantly blunted in HFD mice (SD = 382.77 ± 37.05%, HFD = 208.19 ± 49.32% of baseline value, ANOVA-2, $p = 0.003$).
These results indicate the development of hippocampal leptin resistance in mice fed a high-fat diet, and highlight the general role of this hormone in modulating neuronal activity well beyond areas involved in metabolic homeostasis.

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Poster

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Program#/Poster#: 742.16/JJJ20

Topic: B.07. Synaptic Transmission

Title: Actions of the tryptophan metabolites 3-hydroxy-kynurenine (3HK) and Xanthurenic Acid (XA), on gamma oscillations in the rat hippocampus

Authors: ***T. E. SALT**[1], F. WEISZ[1], R. SCHWARCZ[2], S. A. NEALE[1,3];
[1]UCL Inst. Ophthalmology, London, United Kingdom; [2]Dept. of Psychiatry, Maryland Psychiatric Res. Ctr., Baltimore, MD; [3]Neurexpert Ltd, London, United Kingdom

Abstract: The kynurenine pathway is a major route of tryptophan metabolism, and this pathway may be disturbed in several psychiatric and neurodegenerative disorders (Schwarcz *et al* 2012). 3-Hydroxy-kynurenine (3HK), a molecule arising from tryptophan, is a precursor for several kynurenines, including xanthurenic acid (XA). Interestingly, XA serum levels are reduced in patients with schizophrenia (Fazio *et al* 2015). We have previously shown that both 3HK and XA can reduce hippocampal synaptic transmission, and it is probable that some of the effect of 3HK is attributable to the conversion of 3HK to XA (Neale *et al* 2013; Ngomba *et al* 2015).

Gamma oscillations are thought to be an important biomarker of cognitive functions. We have thus investigated the actions of 3HK and XA on hippocampal *gamma* oscillations in an effort to shed light on the role of these kynurenines in cognitive disorders.
Horizontal *in vitro* slice preparations were made from the brains of adult male Sprague-Dawley rats. Extracellular field recordings were made from the CA3 area of the hippocampus in an interface bath at ~33C. Addition of carbachol (5μM) to the bathing medium resulted in the generation of stable *gamma* oscillations with peak power in the 32-42Hz region. Further addition of XA (0.3-3mM) resulted in a reversible concentration-dependent reduction of the *gamma* power by up to 91±3% (3mM). Addition of 3HK (1mM) also reduced the *gamma* power (by 61±9%) whereas 1mM XA reduced the *gamma* power by only 29±4%. There did not appear to be any significant change in the frequency of the *gamma* power peak under these conditions.
In summary, we have shown that 3HK, the direct metabolic precursor of XA, has similar effects to XA in reducing the power of *gamma* oscillations in the hippocampus. This finding is consistent with the idea that changes in 3HK levels could drive XA levels and thus produce effects on neuronal circuit function, although it does not exclude the possibility that 3HK has direct effects. This may be important in our understanding of how malfunction of the kynurenine pathway can cause changes in circuit function that may be related to pathological changes in cognitive processes.
Fazio F, *et al* (2015). *Scientific Reports* 5, 17799.
Neale SA, *et al* (2013). *Neuropsychopharmacology* 38, 1060-1067.
Ngomba RT, *et al* (2015). *Society for Neuroscience Abstracts*, 571.509.
Schwarcz R, *et al* (2012). *Nat Rev Neurosci* 13, 465-477.

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Poster

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Program#/Poster#: 742.17/JJJ21

Topic: B.07. Synaptic Transmission

Support: CONACyT CB-2013-01 221653

Title: Synaptic effects of social defeat stress: interleukin-6-dependent contribution

Authors: ***E. ESQUIVEL RENDON**[1], J. VARGAS-MIRELES[1], F. MEDINA-GARCIA[1], A. MALDONADO-HERNANDEZ[1], P. ACOSTA-MARES[1], R. CUEVAS-OLGUIN[1], I. GONZALEZ-NATERAS[1], M. MIRANDA-MORALES[1], S. ROSE-JOHN[2], M. ATZORI[1];

[1]Facultad de Ciencias, UASLP, San Luis Potosi, Mexico; [2]Biochem., Christian Albrechts Univ., Kiel, Germany

Abstract: Social stress potentially undermines well-being in humans as well as in many mammalian species. The biological correlates of social stress are not yet completely understood. Recent investigation has underscored the importance of pro-inflammatory cytokines, particularly interleukin-6 (IL-6), as mediators of the effects of stress in the central nervous system (CNS). We considered the possibility that social stress triggers synaptic changes in stress-vulnerable CNS regions in an IL-6-dependent fashion. To test this hypothesis we used GFAP-sgp130Fc (transgenic, TG) mice, a murine model in which IL-6 trans-signaling -a critical mechanism of action IL-6 in the CNS - was blocked. As a model of social stress we determined the effects of Social Defeat Stress protocol (SDS) on wild type (WT, C57BL/6) or TG animals. SDS animals were submitted to a 10-day stress protocol consisting in a 15 min-long sessions in which they were put in contact with a CD1 white mouse, whose aggressive behavior had been determined in previous separate tests. At the end of the protocol we evaluated whether or not experimental animals responded to stress, by comparing the time spent in an area in the proximity of the CD1 mouse vs. the time spent in the same area in the absence of the CD1 mouse. Preliminary experiments showed that while WT stressed animals - as expected - spend considerably less time in the CD1-interaction area, compared to non-stressed animals. Stressed TG animals fail to substantially decrease the time spent in the CD1-interaction area, suggesting that TG mice respond to stress differently from WT mice. We further studied synaptic function of the same animals in their prefrontal cortex, a CNS area that has been shown to be particularly vulnerable to social stress. The synaptic parameters studied were the ratio between α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid-receptor (AMPAR)-, the γ-amino butyric acid type A ($GABA_AR$)-, and the N-methyl-D aspartate-(NMDAR)-mediated component of electrically evoked synaptic currents from visually identified neurons in cortical layer 5 medial prefrontal cortex. Our results showed that SDS decreases the ratio I_{AMPA}/I_{GABA} (0.57 ± 0.11 in non-stressed vs. 0.32 ± 0.02, unpaired Student t-test, d.o.f. = 15) in WT mice but not in TG mice (0.60 ± 0.09 in non-stressed vs. 0.56 ± 0.12, d.o.f. = 18). On the contrary, the ratio between I_{AMPA}/I_{NMDA} remained unchanged regardless of group (WT vs. TG) or treatment (non-stressed vs. stressed). These data suggest that at least part of the behavioral and central effects of social stress are mediated by IL-6 -dependent central trans-signaling.

Disclosures: E. Esquivel Rendon: None. **J. Vargas-Mireles:** None. **F. Medina-Garcia:** None. **A. Maldonado-Hernandez:** None. **P. Acosta-Mares:** None. **R. Cuevas-Olguin:** None. **I. Gonzalez-Nateras:** None. **M. Miranda-Morales:** None. **S. Rose-John:** None. **M. Atzori:** None.

Poster

742. Modulation of Cognition and Behavior I

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Program#/Poster#: 742.18/JJJ22

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant R01 NS040723

UT Arlington Psychology Bridging Funds

Title: Pro-bumetanide treatment reduces long-lasting learning and memory deficits induced by repeated administration of ketamine in neonatal rats

Authors: *B. D. BUTLER[1], M. KHAN[1], R. A. STEVENS[1], S. MUQUEET[1], S. KOKANE[2], P. THAPA[3], F. W. FOSS[3], Q. LIN[2];
[1]Biol., [2]Psychology, [3]Chem., Univ. of Texas At Arlington, Arlington, TX

Abstract: Ketamine (KET), a non-competitive N-methyl-D-aspartate receptor (NMDAR) antagonist is commonly used as a pediatric anesthetic. However, several experimental studies have indicated its involvement in inducing widespread neurotoxicity in the neonatal brain, subsequently leading to long-lasting cognitive and memory deficits that persist into adulthood. Previous studies from our group demonstrated that bumetanide – an Na^+-K^+-$2Cl^-$ cotransporter (NKCC1) antagonist – has the ability to reduce these KET-induced deficits. However, bumetanide does not cross the blood-brain barrier (BBB) efficiently upon systemic injection. Therefore, in this study, we used a derivative form of bumetanide, known as pro-bumetanide (PBUM) which crosses the BBB more efficiently upon systemic administration. In order to investigate the potential neuroprotective effects of PBUM, we co-administered PBUM at varying doses with ketamine or vehicle (VEH). Male and female rat pups, at postnatal day 9, were randomly assigned to the following drug-treatment groups: 6 mg/kg PBUM (6-PBUM)+KET, 12 mg/kg PBUM (12-PBUM)+KET, 18 mg/kg PBUM (18-PBUM)+KET, VEH+KET, and VEH+VEH. About four weeks following drug administration, all groups were tested for spatial learning and memory abilities using the Morris Water Maze (MWM) and Object Location Memory (OLM) task. Consistent with our previous results, the ketamine-only group (VEH+KET) exhibited an increase in time to perform these tests, indicting deficiencies in spatial learning and memory as compared to the control (VEH+VEH). In contrast, the co-treatment groups (n-PBUM+KET) exhibited behavior similar to the control, and outperformed the VEH+KET group, providing evidence that PBUM does demonstrate lasting neuroprotection upon systemic co-administration with ketamine at neonatal ages. This was further corroborated by the OLM results. The n-PBUM+KET groups spent more time in the zone in which the object's location was changed (similar to the VEH+VEH group) while the VEH+KET group spent relatively equal amounts of time in both zones. This indicates that while the n-

PBUM+KET and VEH+VEH groups identified the novel position of the object, the VEH+KET group could not. In conclusion, this study provided evidence that PBUM was effective in preventing cognitive and memory deficits induced by neonatal administration of ketamine. To the best of our knowledge no other research has been performed using a ketamine model to test forms of pro-bumetanide. Thus, these results set precedence for the need to further investigate the potential therapeutic effects of bumetanide derivatives and their underlying biomolecular mechanisms involved.

Disclosures: **B.D. Butler:** None. **M. Khan:** None. **R.A. Stevens:** None. **S. Muqueet:** None. **S. Kokane:** None. **P. Thapa:** None. **F.W. Foss:** None. **Q. Lin:** None.

Poster

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Support: PAPIIT-DGAPA, UNAM Grant IN202414

CONACYT Scholarship 371741 to C. S. P.

CONACYT Grant

Title: Endocannabinoids interact with glucocorticoids in the dorsal striatum: enhancing effects on memory of an aversive motivated task

Authors: ***C. SILLER PÉREZ**[1], E. SOTELO BARRERA[1], A. FUENTES-IBAÑEZ[1], N. SERAFÍN[1], R. PRADO-ALCALÁ[1], P. CAMPOLONGO[2], P. JOSEPH-BRAVO[3], B. ROOZENDAAL[4,5], G. QUIRARTE[1];
[1]Inst. De Neurobiología, UNAM Campus Juriquilla, Queretaro, Mexico; [2]Dept. of Physiol. and Pharmacol., Sapienza Univ. of Rome, Rome, Italy; [3]Dept. de Genética del Desarrollo y Fisiología Mol., Inst. de Biotecnología, UNAM, Cuernavaca, Mexico; [4]Dept. of Cognitive Neurosci., Radboud Univ. Med. Ctr., Nijmegen, Netherlands; [5]Donders Inst. for Brain, Cognition and Behaviour, Radboud Univ. Nijmegen, Nijmegen, Netherlands

Abstract: Glucocorticoids, released during arousing experiences, enhance the consolidation of memory processing. Recent findings indicated that glucocorticoids interact with the endocannabinoid system of the hippocampus or the amygdala in modulating memory consolidation. Previously, we have shown that corticosterone administration into the dorsal striatum also enhances memory of inhibitory avoidance training. In the striatum, the cannabinoid

receptor type 1 (CB1R) is highly expressed but it remains unknown if endocannabinoids cooperate with glucocorticoids to enhance striatal-dependent memory. Our objective was to evaluate if there is a striatal glucocorticoid-endocannabinoid interaction that modulates memory consolidation of an aversive experience. Male Wistar rats, with bilateral cannulae in the anterodorsal striatum, were trained on an inhibitory avoidance task and had a retention test 48 h later. We found that the CB1R antagonist AM251 (0.28 or 0.56 ng/µl) administered into the striatum immediately after the inhibitory avoidance training impaired memory consolidation. In contrast, the CB1R agonist WIN55-212 (50 or 100 ng/µl) enhanced memory consolidation, and this effect was blocked by systemic administration of the corticosterone-synthesis inhibitor metyrapone (50 mg/kg, ip) given 90 min before training. Additionally, a low dose of AM251 (0.28 ng/µl) administered immediately posttraining blocked the memory-enhancing effect of posttraining corticosterone administered systemically (3 mg/kg, ip) or of corticosterone (20 ng/0.5 µl) infused directly into the dorsal striatum. Our findings provide evidence on the broad interaction of endocannabinoids with glucocorticoids that modulate memory, now assessed in the striatum; suggesting that the endocannabinoid system is recruited by glucocorticoids to enhance memory consolidation of an emotional aversive training experience. We thank the excellent technical support from Cristina Medina, Martín García, Leonor Casanova, and Lourdes Lara. Work supported by PAPIITDGAPA, UNAM IN202414, and CONACYT (Grant and Scholarship 371741 to C.S.P).

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Poster

742. Modulation of Cognition and Behavior I

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Program#/Poster#: 742.20/JJJ24

Topic: H.01. Animal Cognition and Behavior

Support: Colombian Science, Technology and Innovation Department (Colciencias) Grant 621-2014-110165843270

Title: Trichostatin A prevents stress-induced spatial memory impairment

Authors: V. VARGAS-LÓPEZ[1], *A. MÚNERA[2];
[1]Physiol., Univ. Nacional de Colombia, Bogotá D.C., Colombia; [2]Univ. Nacional De Colombia, Bogotá, Colombia

Abstract: Acute stress induced before spatial training impairs long-term memory and it is related to decreased dendritic spine density, diminished kinase activity, and reduced expression neural cell adhesion molecule (NCAM), brain-derived neurotrophic factor (BDNF), and activity-regulated cytoskeleton-associated protein (Arc) in the hippocampus. Epigenetic mechanisms involved in such effect has not been yet study; but, since spatial training and intense stress have opposite effects on histone acetylation balance, it is conceivable that disruption of such balance may underlie acute stress-induced spatial memory consolidation impairment. In this study, we tested the effectivity of the histone deacetylase inhibitor Trichostatin A (TSA) to prevent the deleterious effect of acute restrain stress on long-term spatial memory in adult male Wistar rats. We found that animals stressed before training in the Barnes maze showed impaired long-term memory, in association with increased plasma corticosterone levels, and decreased histone H3 acetylation levels in the dentate gyrus of the hippocampus and the prelimbic cortex, as compared to non-stressed animals. Remarkably, these effects were prevented by intraperitoneal administration of TSA (1mg/kg) immediately after the spatial training. The aforementioned results are consistent with reports showing that histone deacetylases inhibition can prevent stress-induced anxiety, somatic hypersensitivity, and visceral pain, and supports the hypothesis that deleterious effects of stress on memory involve altered histone acetylation balance.

Disclosures: **V. Vargas-López:** None. **A. Múnera:** None.

Poster

742. Modulation of Cognition and Behavior I

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Support: NS045260

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Title: The endocannabinoid 2-AG mediates a novel form of presynaptic plasticity at a critical relay in hippocampal circuitry

Authors: *W. WANG[1], B. H. TRIEU[1], Y. JIA[1], K.-M. JUNG[1], C. A. KARSTEN[1], C. B. MERRILL[1], K. MACKIE[2], C. M. GALL[1,3], D. PIOMELLI[1,4,5,6], G. LYNCH[1,7];
[1]Dept. of Anat. and Neurobio., Univ. of California Irvine Dept. of Anat. and Neurobio., Irvine, CA; [2]Dept. of Psychological and Brain Sci. and Gill Center, Indiana Univ. Bloomington, Bloomington, IN; [3]Dept. of Neurobio. and Behavior, Univ. of California, Irvine, Irvine, CA; [4]Dept. of Pharmacology, Univ. of California, Irvine, Irvine, CA; [5]Dept. of Biol. Chemistry, Univ. of California, Irvine, Irvine, CA; [6]Drug Discovery and Development, Inst. Italiano di Tecnologia, Genoa, Italy; [7]Dept. of Psychiatry, Univ. of California, Irvine, Irvine, CA

Abstract: The endocannabinoid 2-arachidonoyl-sn-glycerol (2-AG) modulates synaptic function at many loci throughout the brain with effects generally mediating a dampening of transmission. There is good evidence that 2-AG is produced, on demand in dendritic spines by metabotropic glutamate receptor (mGluR)-mediated activation of the synthetic enzyme diacylglycerol lipase-α (DGL) and acts upon presynaptic CB_1 receptors to depress release. The present studies re-evaluated endocannabinoid actions at lateral perforant path (LPP) innervation the dentate gyrus and identified a novel form of 2-AG-dependent signaling that mediates an enduring enhancement of transmission that entails increases in neurotransmitter release. In studies of hippocampal slices from rats and mice, we found that high frequency stimulation of the LPP induces long term potentiation (LTP) that is blocked by CB_1 receptor antagonism and absent in CB_1 knockouts; the same manipulations had no effect on the magnitude of LTP in the adjacent medial perforant path or in field CA1. Results indicate that 2-AG mediates the endocannabinoid-dependent LTP (ecLTP) in the LPP: It is blocked by DGL inhibition but enhanced by antagonism of 2-AG degradation whereas manipulation of anadamide degradation had no effect. Mechanisms of ecLTP include activities in both pre- and postsynaptic compartments. Induction relies upon mGluR and NMDA receptors, and changes in postsynaptic calcium, whereas expression entails a depression of paired pulse facilitation indicating increased neurotransmitter release. Evidence for ecLTP in the LPP suggests that memory encoding that relies upon this system is also endocannabinoid, and 2-AG, dependent. We tested this using an odor discrimination task known to rely upon the lateral entorhinal to dentate projection. In rats, odor discrimination learning was impaired by CB_1 antagonism but facilitated by treatments that increase 2-AG content. Together these results demonstrate that the encoding of information carried by a principal hippocampal afferent involves a novel, endocannabinoid-dependent form of synaptic plasticity.

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Poster

742. Modulation of Cognition and Behavior I

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Program#/Poster#: 742.22/JJJ26

Topic: H.01. Animal Cognition and Behavior

Support: BSI

CPUB

SRG

FRG

Title: Interactions between progesterone & tropisetron in the modulation of cognitive behavior in female rats.

Authors: J. HASSELL, M. MAZENKO, *S. S. MASWOOD;
Biol., Millersville Univ., Millersville, PA

Abstract: The neurotransmitter serotonin (5-HT) is involved in the modulation of cognitive functions. The precise mechanism of action of the 5-HT system in the facilitation of cognitive functions has not been clearly identified. However, compounds such as tropisetron that act as an antagonist at the 5-HT_3 receptor enhance cognition. Similarly in female rodents, priming with estrogen and/or progesterone enhances cognition in several behavioral models including the object recognition task. The object recognition task is a model of cognition in rodents in which the natural tendency of rats to explore novel aspects of the environment is utilized. Rats spend more time exploring the novel object, suggesting that rats recognize previously explored objects. Earlier we reported that the 5-HT_3 receptor antagonist, tropisetron, enhances object recognition in regularly cycling intact female rats. Interestingly, both estrogen and progesterone also modulate the functioning of 5-HT_3 receptors by acting as antagonists at this receptor. Since estrogen, progesterone and tropisetron all act as antagonists at the 5-HT_3 receptor, we hypothesized that the cognitive enhancing effects of tropisetron would be further accentuated in ovariectomized female rats primed with either estrogen or progesterone.
Contrary to expectation, we did not find a synergistic effect between estrogen and tropisetron in the object exploration task. Our preliminary data however, show that progesterone and tropisetron may act synergistically to enhance cognition. Since we looked at a single dose of progesterone and tropisetron, we are currently evaluating multiple doses of progesterone and tropisetron in ovariectomized Sprague-Dawley female rats using the object recognition task as a model of cognition. Findings from this study will help us further understand the interaction between the serotonergic system and female hormones in the modulation of cognition.

Disclosures: **J. Hassell:** None. **M. Mazenko:** None. **S.S. Maswood:** None.

Poster

742. Modulation of Cognition and Behavior I

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Program#/Poster#: 742.23/JJJ27

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant T32 ES007051

CCHMC Arnold W. Strauss Fellow Award

Title: Neurotoxic and cognitive effects of neonatal (+)-methamphetamine: Role of reactive oxygen species (ROS)

Authors: ***S. A. JABLONSKI**[1], K. LASANCE[2], L. LEMEN[2], S. J. THOMAS[3], S. C. SANCHEZ[3], M. ASCHNER[4], M. T. WILLIAMS[1], C. V. VORHEES[1];
[1]Neurol., Cincinnati Children's Hosp. Med. Ctr., Cincinnati, OH; [2]Radiology, Univ. of Cincinnati Col. of Med., Cincinnati, OH; [3]Clin. Pharmacol., Vanderbilt University, Sch. of Med., Nashville, TN; [4]Mol. Pharmacol., Albert Einstein Col. of Med., New York, NY

Abstract: Rats treated with (+)-methamphetamine (MA) during neonatal development (equivalent to third trimester brain development in humans) results in deficits in allocentric learning and memory (L&M) in the Morris water maze (MWM), egocentric learning in the Cincinnati water maze (CWM), working and reference memory impairments in the radial water maze, and in contextual fear conditioning. These L&M deficits are substantial, stable, and larger than in adult animals (Vorhees et al., 2007). However, it remains to be elucidated what mechanisms mediate the developmental MA-induced cognitive deficits in the absence of dopamine-related effects that are found in adult rodents after MA exposure. Neonatal MA-exposed rats also exhibit exaggerated hyperactivity in response to the D_1 agonist SKF-82958, reduced hyperactivity to the NMDA antagonist MK-801, and mild under-response to D_2 autoreceptor agonist, quinpirole, but no changes in response to serotonergic agonists. Because prenatal exposure to MA increases reactive oxygen species (ROS) in adult rats, we tested the involvement of ROS after neonatal MA exposure in two ways: by measuring F_2 isoprostanes (F_2-IsoPs) and by pretreatment with the spin trapping agent alpha-phenyl-N-tert-butyl nitrone (PBN) prior to each dose of MA. There was no change in F_2-IsoPs in striatum or hippocampus after the first dose on P6 or last dose on P15. Nor did PBN attenuate MA-induced L&M deficits. We investigated striatal D_1 receptors using micro PET/CT imaging and the D_1 ligand, TISCH, after treated rats were adults. We found no effects of treatment. We also examined brain oxygen

activity using 2-fluoro-2-deoxyglucose (FDG) and found no change after challenge with the D_1 agonist SKF-82958. In sum, third trimester-equivalent MA exposure (P11-20 or P6-15) in rats leads to long lasting L&M impairments and changes in dopaminergic and glutamatergic receptor function, but not in ROS production, or changes in D_1 receptor binding or metabolic activity. Hence, the exact mechanism of MA-induced developmental effects remains to be elucidated.

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Poster

742. Modulation of Cognition and Behavior I

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Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant AA017359

Title: Gonadal hormones modulate ethanol induced memory deficit in adolescent female rats

Authors: J. A. TRAVIS[1], K. ISHIWARI[2], ***R. SIRCAR**[1,3];
[1]The City Col. of New York, New York, NY; [2]Res. Inst. on Addictions, Univ. at Buffalo, Buffalo, NY; [3]Psychiatry & Behavioral Sci., Albert Einstein Col. of Med., Bronx, NY

Abstract: Earlier, our lab has reported that ethanol treatment in pre-pubertal adolescent male and female rats causes hippocampus-associated memory deficit. Whether ethanol affects hippocampus related memory function in post-pubertal rats is not known. In this study, we investigated the effects of ethanol on hippocampus-associated and amygdala-related cognitive functioning in post-pubertal adolescent male and female rats. Post-pubertal rats were administered a single injection of ethanol (2 g/kg) intraperitoneally or equivalent volumes of deionized water. Some female rats were ovariectomized and given hormonal supplementation (estrogen and/or progesterone). Additional controls included sham-operated vehicle-treated animals. All rats were trained in the fear conditioning paradigm. Twenty-four hours later, all experimental animals were tested for: (i) contextual fear conditioning in the same training chamber, and (ii) cued fear memory in a modified test chamber. For each rat, freezing during contextual and cued fear conditioning tasks were recorded. Acute ethanol-treatment in intact post-pubertal female rats showed significant disruptions in hippocampus-related contextual memory but not amygdala-associated cued fear memory. Post-pubertal male rats did not show any ethanol-induced memory deficit. There was significant effect of estrous cyclicity on ethanol-induced behavior in intact female rats. Exogenously administered estrogen with or without

progesterone altered the sensitivity of ethanol-induced memory impairment in ovariectomized post-pubertal female rats. Together, these data suggest that female gonadal hormones play an important role in modulating ethanol-induced memory impairment in post-pubertal animals.

Disclosures: **J.A. Travis:** None. **K. Ishiwari:** None. **R. Sircar:** None.

Poster

742. Modulation of Cognition and Behavior I

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Program#/Poster#: 742.25/JJJ29

Topic: H.01. Animal Cognition and Behavior

Support: R01NS089575

Title: Cannabinoid receptor antagonist AM251 reduces radiation-induced cognitive decrements

Authors: ***V. K. PARIHAR**, A. LILAGAN, A. SYAGE, S. F. KWOK, N. N. CHMIELEWSKI, M. M. ACHARYA, J. E. BAULCH, C. L. LIMOLI;
Dept. of Radiation Oncology, Univ. of California, Irvine, CA

Abstract: There are now more than 10 million cancer survivors in the United States. With these numbers, chronic sequelae that result from cancer therapy have become a major health care problem. Although radiation therapy of the brain has improved cancer cure rates, learning disorders and memory deficits are a common consequence of this therapy. As exposure to ionizing radiation results in enhanced depressive- and anxiety-like behaviors and memory dysfunction, it is believed that the inhibition of hippocampal neurogenesis represents an underlying mechanism. Here, we investigated the effects of the cannabinoid receptor antagonist AM251 on radiation-induced cognitive decrements and reduced neurogenesis 1 and 3 months following exposure. To validate the functional significance of CB1 receptor blockade, 6 month old mice were first subjected to head only irradiation (9 Gy) then given 4 consecutive daily doses of AM251 (1 mg/kg) every month for 3 months. Learning and memory was assessed by novel object recognition (NOR) and Object in Place (OiP) memory tasks, while the Elevated Plus Maze Test (EPM) was used to quantify anxiety-like behavior. Preliminary studies have indicated a potential neurocognitive benefit of AM251 treatment in irradiated mice. Interestingly, quantification of BrdU+ cells and the number of doublecortin (DCX) positive newly born neurons in the subgranular zone-granule cell layer has revealed that AM251 enhances neurogenesis and cell proliferation compared to the control group ($p<0.05$). Our findings suggest that certain radiation-induced sequelae can be reduced by AM251 treatment, possibly through a mechanism involving enhanced neurogenesis.

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Poster

743. Learning and Memory: Aging - Other Areas

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Program#/Poster#: 743.01/JJJ30

Topic: H.01. Animal Cognition and Behavior

Support: NIH R01AG043972

P30NS47466

P30DK056336

P30DK079626

Title: Sex differences in the metabolic and cognitive effects of ghrelin signalin

Authors: ***I. KADISH**[1], M. SHARPE[1], T. VAN GROEN[1], A. PATKI[2], T. R. NAGY[3], D. B. ALLISON[4];
[1]Dept Cell, Developmental and Integrative Bio, [2]Dept Biostatistics, [3]Dept Nutr. Sci., [4]Sch. of Publ. Health, Nutr. Obesity Res. Ctr., Univ. of Alabama Birmingham, Birmingham, AL

Abstract: Environmental interventions that affect rate of aging and life span tend to have a greater effect in one sex than the other. Whereas studies have been done on the effects of sex differences in many neurological diseases, e.g., Alzheimer's disease and autism, the role that gender plays in metabolic diseases is much less clear. Ghrelin (a gut hormone), via hypothalamic circuits, is involved in hunger and meal initiation causing *perceived* negative energy balance even in eucaloric conditions. Interoceptive cues caused by ghrelin are likely similar to those produced by caloric restriction. In previous studies we have shown that long term treatment with ghrelin agonist improves cognition and glucose handling in aging male mice. In the current study we tested the hypothesis that ghrelin agonist treatment in female mice will have more pronounced positive effects on metabolism and cognition than in male mice. Female C57BL/6J mice at two months of age were randomly assigned into 4 groups (n=20/group) on the basis of weight matching. One group (control) received *ad libitum* access to food while the other group (ghrelin) received the same amount of diet consumed by the control group and 30 mg/kg of synthetic ghrelin agonist (LY444771) daily, the third and fourth groups underwent ovariectomy (OVX control and OVX-ghrelin). Food consumption and body weights were measured weekly. At 10 months of age an oral glucose tolerance test was performed. At 12 months of age all

groups were submitted to a battery of cognitive and behavioral tests, followed by an imaging procedure (QMR) and indirect calorimetry. Biochemistry and immunohistochemistry analysis were carried out to assess the changes in brain tissue and plasma. Ghrelin agonist treatment improved glucose tolerance in both ghrelin agonist treatment groups to compare with the control groups. There was significant ($p<0.0001$) increase in energy efficiency in ghrelin treated animals. Cognition was improved ($p<0.04$) in OVX ghrelin treated group to compare with OVX control, but did not significantly influence the cognitive outcome of 12 month old intact mice. Insulin signaling was improved in hippocampus and inflammation was attenuated in white matter and hypothalamus in ghrelin agonist treated mice. In conclusion, to compare with male mice, long term ghrelin agonist treatment in female mice significantly increased energy efficiency in intact and OVX and improved cognition in middle age OVX mice.

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Poster

743. Learning and Memory: Aging - Other Areas

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Topic: H.01. Animal Cognition and Behavior

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Swedish Brain Power

StratNeuro

Karolinska Institutet Research Foundations

Swedish Brain Foundation

Karolinska DPA

Title: Nogo receptor 1 overexpression in forbrain neurons alters dopamine release kinetics

Authors: *E. S. ARVIDSSON, T. E. KARLSSON, A. T. BRODIN, K. WELLFELDT, A. JOSEPHSSON, L. OLSON;
Karolinska Institute, Dept. of Neurosci. (, Stockholm, Sweden

Abstract: The ability to learn from experience and to store memories is fundamental to life. Losing these abilities, as in Alzheimer's disease, is devastating. To implement the required structural synaptic re-organizations needed to permanently embed novel memories in the brain, a complex and precisely organized molecular machinery consisting of plasticity-enhancing and plasticity-inhibiting systems needs to be activated. We previously discovered that increased neural activity is linked to rapid down-regulation of Nogo receptor 1 (NgR1). To test if such regulation is important for the formation of lasting memories, we generated MemoFlex mice, with inducible overexpression of NgR1 in forebrain neurons. While these mice have normal LTP and, under undemanding circumstances, normal 24 hr memory, formation of lasting memories is severely impaired. However, when transgenic NgR1 expression is turned off in these mice (by doxycycline), the ability to form lasting memories is restored. We also found that the presumed structural synaptic reorganization needed to form permanent engrams occurs during the first week after experiencing a memory-causing event. The aim of this study is to further investigate how Nogo-signaling affects neurotransmitter release. Because dopamine signaling has been implicated in regulating plasticity and memory, we used electrochemical measurements with amperometry to allow *in vivo* quantification of dopamine with a very high temporal and spatial resolution in NgR1 overexpressing mice. Initial results revealed severe impairment of KCl-induced dopamine release in dorsal striatum in overexpressing, compared to littermate controls. Ongoing work focuses on the mechanisms behind this effect. We will determine if turning off the NgR1 transgene might result in normalization of DA kinetics and if DA release kinetics are altered in mice lacking NgR1.

Disclosures: E.S. Arvidsson: None. **T.E. Karlsson:** None. **A.T. Brodin:** None. **K. Wellfeldt:** None. **A. Josephsson:** None. **L. Olson:** None.

Poster

743. Learning and Memory: Aging - Other Areas

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 743.03/JJJ32

Topic: H.01. Animal Cognition and Behavior

Support: NIH R37AG008796

NIH R01AG017139

NIH T32AG020506

Title: Aging decreases intrinsic excitability within layer II/III principal cells of the lateral entorhinal cortex

Authors: *C. LIN, M. OH, J. DISTERHOFT;
Dept. of Physiol., Northwestern Univ., Chicago, IL

Abstract: Aging is often associated with a decline in hippocampus-dependent learning and memory. Numerous studies have investigated the neurobiological mechanisms that underlie aging-related learning and memory impairments. Previous research has shown that within the CA1 region of the hippocampus, a decrease in intrinsic excitability within pyramidal neurons underlies learning deficits within aged animals. The current project focuses on determining whether intrinsic excitability within the principal neurons of the entorhinal cortex also decreases with age and whether these changes may also contribute to age-associated learning deficits observed. The entorhinal cortex relays information from cortical regions to the hippocampus and is highly susceptible to aging-related changes. Specifically, the lateral portion of the entorhinal cortex (LEC) been suggested to support hippocampus-dependent temporal associative learning. Therefore, whole cell current clamp recordings were performed on the principal neurons of layer II/III of the LEC from young adult (3-6 month old) and aged (29-32 month old) F1 F344xBN hybrid rats. Within layer II, these are the fan cells and within layer III, the recordings were made from pyramidal neurons. Measures of intrinsic excitability include accommodation and postburst afterhyperpolarization (AHP). Our preliminary results indicate that fan and pyramidal neurons from aged animals are less excitable relative to their younger counterparts. This decrease in excitability manifests as an increase in accommodation and an increase in the postburst AHP. The results are similar to previous data from the CA1 region of the hippocampus and suggest LEC intrinsic excitability may also be a measure and mechanism supporting learning. Identification of these changes in aging will point to a potential target for future therapeutics in alleviating aging-related learning and memory deficits.
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Disclosures: C. Lin: None. **M. Oh:** None. **J. Disterhoft:** None.

Poster

743. Learning and Memory: Aging - Other Areas

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Program#/Poster#: 743.04/JJJ33

Topic: H.01. Animal Cognition and Behavior

Support: by NASA Grants NNX13AB73G and NNX16AE06G

Title: Age as a risk factor for the disruption of cognitive performance by exposure to the types of radiation encountered on exploratory class missions to other planets

Authors: *B. M. RABIN[1], B. SHUKITT-HALE[2], K. L. CARRIHILL-KNOLL[1], M. G. MILLER[2], K. A. BARTON[1];
[1]Univ. Maryland Baltimore County, Baltimore, MD; [2]Human Nutr. Res. Ctr. on Aging, USDA-ARS, Boston, MA

Abstract: Exposure to the types of radiation encountered in space (particles of high energy and charge [HZE particles]) produces changes in neurocognitive performance similar to those observed in the aged organism. As such, it is possible that there would be an interaction between the effects of exposure to HZE particles and the effects of aging. Previous research using ^{56}Fe particles has shown an interaction between age of irradiation and the dose needed to produce a deficit in cognitive performance, such that doses of HZE particles that did not affect the performance of younger subjects affected the performance of older ones. The present experiments were designed to evaluate the generality of the relationship between age of irradiation and the dose needed to disrupt cognitive performance as a function of the characteristics of the specific particle. Male F-344 rats between 2 and 16 months of age were exposed to ^{48}Ti, ^{16}O, and ^{4}He particles at the NASA Space Radiation Laboratory at Brookhaven National Lab. Cognitive performance was evaluated by testing performance on an ascending fixed-ratio operant task. In this task the subject must make an increasing number of responses in order to obtain a reward (food pellet). This task measures the motivation of the organism to work for reinforcement and the responsiveness of the organism to changes in environmental contingencies, including changes in reinforcement contingencies. Overall, the results indicated that for the higher linear energy transfer (LET) particles (^{56}Fe and ^{48}Ti; which produce greater tissue destruction along the particle track) a lower dose was needed to disrupt cognitive performance in the older subjects. Conversely, the available results suggest that a similar relationship may not be observed for the lower LET particles (^{16}O and ^{4}He) which produce less tissue destruction along the particle track). These results suggest that the effectiveness of specific particles in disrupting cognitive performance may, under some conditions, vary as a function of the age of the organism at the time of irradiation and the LET of the specific particle.
Acknowledgments: This research was supported by NASA Grants NNX13AB73G and NNX16AE06G.

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Poster

743. Learning and Memory: Aging - Other Areas

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Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 743.05/JJJ34

Topic: H.01. Animal Cognition and Behavior

Support: Regis URSC Grant

Title: The effects of cognitive enrichment and exercise on age-related cognitive decline in rats

Authors: *M. E. BASHAM;
Neurosci. Program, Regis Univ., Denver, CO

Abstract: A decline in cognitive abilities is an inevitable part of aging. However, cognitive activity during one's lifetime can create a cognitive reserve that partially mitigates this cognitive decline. Thus, cognitive enrichment programs aimed at older adults have been developed in an attempt to delay, or even reverse, normal, age-related cognitive decline. Overwhelmingly, the animal research into mitigating age-related cognitive decline using cognitive interventions involves cognitive enrichment beginning early in life and often persisting into adulthood. These animal models are analogous to the human experience of education beginning early in life. Very few animal models present cognitive enrichment later in life, analogous to the brain training programs currently offered to older adults. Moreover, many of the animal models confound cognitive enrichment and exercise, making it difficult to attribute cognitive gains to cognitive activity rather than physical activity. Therefore, the present study administered either cognitive enrichment or exercise to already aged rats and then compared their cognitive performance to aged controls. In a water maze task, exercised aged rats swam faster and shorter distances to a hidden platform than control rats, but rats exposed to cognitive enrichment late in life did not perform better than controls. Surprisingly, exercised aged rats spent less time searching in the appropriate quadrant during probe trials than either control rats or rats exposed to cognitive enrichment late in life. These findings suggest that cognitive enrichment presented exclusively later in life will not mitigate normal age-related cognitive decline.

Disclosures: M.E. Basham: None.

Poster

743. Learning and Memory: Aging - Other Areas

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Program#/Poster#: 743.06/JJJ35

Topic: H.01. Animal Cognition and Behavior

Support: RI 6039 Faculty Seed Grant

NIA P01 AG022550

Title: The effects of a combination dietary intervention of caloric restriction and curcumin on normally aged C57BL/6 mice

Authors: *M. SARKER[1], S. FRANKS[2], N. SUMIEN[2], M. J. FORSTER[2];
[1]UNT Hlth. Sci. Ctr., Fort Worth, TX; [2]Univ. of North Texas Hlth. Sci. Ctr., Fort Worth, TX

Abstract: Our main hypothesis was that a combination treatment of dietary curcumin and CR will improve age associated neurobehavioral dysfunction better than either treatment alone via anti-inflammatory and antioxidant mechanisms. To determine whether or not combining these two interventions would result in additive or synergistic benefits, dietary curcumin and caloric restriction were tested for functional end points, either alone or in combination, in late middle age (MAG) (15 months) and early senescent (AG) (20 months) C57BL/6J male and female mice. Mice were assigned in groups to receive: (i) base diet ad libitum (AL), (ii) weight stable caloric restriction (CR), (iii) curcumin in the base diet (7200 mg/kg diet) (CURAL) or (iv) curcumin plus CR (CURCR). After 8 weeks of treatment, mice underwent a behavioral battery that tested for cognitive and psychomotor function. The final blood draw for assessment of inflammation and oxidative stress was at 16 weeks of dietary treatment. Curcumin overall improved visual acuity for both MAG males and females as well as AG males compared to their age matched control. There was no effect of dietary treatment on bridge walking for females, which is indicative of cerebellum functioning, compared to their age-matched control. CURCR and CR MAG and AG males however outperformed their age matched control on the bridge walking test. All three dietary interventions had significant effects on pro and anti-inflammatory cytokines, KC-GRO concentration was significantly decreased in CR and CURCR in the middle aged males and females group and was correlated to body weight; TNF-α concentration was significantly decreased in both CURAL and CURCR in middle aged males and there was a significant decrease in C-reactive protein in aged females under CURCR. MAG CURAL and AG CR males had lower levels of oxidative stress compared to baseline measures. Based on results from the motor tests and blood based biomarkers and earlier data on cognitive and other psychomotor function, dietary curcumin can be used as a CR mimetic for certain components of healthy functional aging but the benefits may not entirely be related to its anti-inflammatory and anti-oxidant effects. Future studies should include different doses and a tapered down caloric restriction regimen.

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Poster

743. Learning and Memory: Aging - Other Areas

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Program#/Poster#: 743.07/JJJ36

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant AG046266

Title: Sex differences in cognition, emotional reactivity, and motor ability in gonadally-intact middle-aged marmosets (Callithrix jacchus)

Authors: *N. GERVAIS[1], K. P. WORKMAN[1], M. LACLAIR[2], A. LACREUSE[1];
[1]Psychological and Brain Sci., [2]Neurosci. and Behavior, Univ. of Massachusetts Amherst, Amherst, MA

Abstract: Sex differences in cognition are well documented. Women outperform men on measures of perceptual speed and verbal abilities, while men outperform women on tests of spatial processing. Robust sex differences also exist in stress responses. However, it is unclear how these sex differences change over time and whether males and females follow different trajectories of age-related cognitive decline. Studies in nonhuman primate models can help resolve this issue. The common marmoset (*Callithrix jacchus*) is a New World primate with a short lifespan that can perform complex cognitive tasks in computerized settings that are comparable to those used with humans. The present study is part of a longitudinal project aimed at determining whether males and females follow different trajectories of cognitive aging. This report focuses on sex differences at study entry. Thirteen marmosets (7 females), aged 4-6 years were tested on a comprehensive battery of tasks assessing cognitive function, motor skills and emotional reactivity. For cognition, monkeys were initially trained on a simple visual discrimination problem, followed by reversal learning using the Cambridge Neuropsychological Test Automated Battery (CANTAB). They also performed the Hill-and-Valley task as a measure of fine motor skills. To assess emotional reactivity, each marmoset was separated from their colony for 7 hours. Behavioral assessments, which involved recording the occurrence of approximately 25 behaviors, occurred a total of 6 times: immediately before separation, 3 times during separation, immediately after separation, and 24-hr later. No sex difference was found for simple discrimination, but males tended to perform better than females on the reversal learning task. No sex difference was observed in motor skills. During separation from the colony, females were more reactive than males, as indicated by more agitated locomotion, and vocalizations. Together, these findings expand upon previous studies and demonstrate sex differences in reversal learning and emotional reactivity in gonadally-intact middle-aged marmosets. As the study progresses, we should be able to determine the neural correlates of these sex differences and how they may change with aging.

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Poster

743. Learning and Memory: Aging - Other Areas

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Program#/Poster#: 743.08/JJJ37

Topic: H.01. Animal Cognition and Behavior

Title: Novel object recognition in aging mice - a critical evaluation of analytical methods in the comparison of C57BL/6 WT and NLRP3 knockout mice.

Authors: *A. TRASCHUETZ, S. SCHWARTZ, M. T. HENEKA;
AG Klinische Neurowissenschaften, Universitätsklinikum Bonn, Bonn, Germany

Abstract: Beside the assessment of spatial learning in various mazes, novel object recognition tasks have become the most widely applied behavioral tests of learning in both rats and mice. With ample evidence that they probe hippocampus-dependent memory formation, these tests have become gold standards in experiments investigating mechanisms or interventions in rodent models of neurodegeneration. For example, novel object tasks have been used to show that the development of Alzheimer's disease-like pathology and cognitive impairment in APP/PS1 mice depends on the NLPR3 inflammasome (Heneka et al, Nature, 2013).
Here, we investigated the value of a novel object recognition task with a 24h intersession interval in probing the impairment of hippocampal memory functions in aging C57BL/6 Wildtype and NLRP3ko mice. Suprisingly, we discovered that even when established protocols are applied, the results of a novel object task can be manipulated by an arbitrary selection of analytical parameters. Young mice (3 months) maintain their novel object preference during the whole 10-minute-probe session. Older mice (9 and 12 months), however, demonstrate a significant preference for the novel object only within the first 1-2 minutes of the test session. This indicates that the behavioral phenotype of older mice not only depends on memory function, but also on altered novel object preference per se. Despite the use of a cutoff for the total exploration time to account for differences in exploratory activity (Leger et al., Nature Protocols, 2013), memory performance of older mice appears to be severely impaired for total exploration times longer than 5 seconds.
Developping an unbiased analytical approach, we show that object recognition memory in aging mice is only mildly impaired in aging. However, we demonstrate that this impairment nevertheless occurs at the age of just 9 months and is mediated by NLRP3-dependent inflammation. Caution should be applied when using or interpreting results of novel object recognition tasks in aging mice.

Disclosures: **A. Traschuetz:** None. **S. Schwartz:** None. **M.T. Heneka:** None.

Poster

743. Learning and Memory: Aging - Other Areas

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Program#/Poster#: 743.09/JJJ38

Topic: H.01. Animal Cognition and Behavior

Support: HHMI

Title: Delivery of osteocalcin in the dentate gyrus enhances cognitive functions and ameliorates age relate memory loss

Authors: *S. KOSMIDIS[1], A. POLYZOS[3], L. KHRIMIAN[2], G. KARSENTY[1], E. KANDEL[1]; [1]Neurosci., [2]Genet. and Develop., Columbia Med. Ctr., New York, NY; [3]Mol. Biol. Div., Biomed. Res. Fndn. of the Acad. of Athens (BRFAA), Athens, Greece

Abstract: The interplay between brain and body metabolism is now beginning to be studied at the molecular level. Recent findings pinpoint osteocalcin (Ocn) as a hormone produced by bone that affects cognitive functions. However, the precise molecular and functional consequences of osteocalcin in the brain, are largely unknown. Here we perform local injection of recombinant osteocalcin in the hippocampal areas that accumulate Ocn and assess its cognitive effects. We identify an osteocalcin-dependent molecular pathway associated with memory, and pinpoint its mechanism of action through the RbAp48 protein. Rbap48 deficiency has been previously described as a key component of Age Related memory loss. We present evidence that OCN acts to ameliorate cognitive symptoms of normal aging and to restore age related memory loss by increasing the RbAp48 /CREB protein levels in the dentate gyrus.

Disclosures: **S. Kosmidis:** A. Employment/Salary (full or part-time): Columbia Medical Center, HHMI. **A. Polyzos:** A. Employment/Salary (full or part-time): BRFAA. **L. Khrimian:** A. Employment/Salary (full or part-time): Columbia Medical Center. **G. Karsenty:** A. Employment/Salary (full or part-time): Columbia Medical Center. **E. Kandel:** A. Employment/Salary (full or part-time): Columbia Medical Center, HHMI.

Poster

743. Learning and Memory: Aging - Other Areas

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Title: A multiple microinfarction based model for vascular dementia in aged rats

Authors: *P. VENKAT, M. CHOPP, J. CHEN;
Neurol. Res., Henry Ford Hosp., Detroit, MI

Abstract: Background and purpose: Vascular Dementia (VaD) affects cognition and memory. VaD accounts for nearly 20% of all dementia patients and is prevalent among the older population. In this study, we investigated a multiple microinfarction (MMI) model in aged male Wistar rats (16-18months), and assessed the consequent cognitive decline and white matter (WM) damage. **Materials and methods:** Male aged Wistar rats (16-18 months) were subjected to MMI (500, 70-100μm cholesterol crystals injected into the internal carotid artery, n=6/group) and cognitive deficits were evaluated 4 weeks later. Rats were sacrificed 4 weeks after MMI for immunohistochemical analysis. **Results:** The MMI model in aged rats induces vascular dementia with significant ($p<0.05$) short term memory loss (novel object recognition test), long term memory loss (odor test), anxiety-like behavior (open field evaluation), and impaired spatial learning and memory (Morris water maze test) at 4 weeks after MMI compared to aged control rats. MMI induces significant sensorimotor deficits that persist until at least 4 weeks after MMI. Compared to aged control rats, MMI significantly ($p<0.05$) decreases axon and myelin density, oligodendrocyte progenitor cells and oligodendrocytes numbers, and decreases synaptic protein expression in the WM tracts of the corpus callosum and striatal WM bundles. Golgi staining indicates that in comparison to aged control rats, aged-MMI rats have significantly ($p<0.05$) decreased neurite branching and cortical and hippocampal spine density. MMI in aged rats significantly damages hippocampal neurons. MMI in aged rats significantly induces water channel dysfunction indicated by decreased Aquaporin-4 (AQP-4) expression around blood vessels in the striatum. This AQP-4 loss around vessel significantly ($p<0.05$) correlates with cognitive deficits. **Conclusions:** The MMI model in aged rats induces significant cognitive impairment and WM/axonal damage primarily in the WM tracts of the corpus callosum and

striatal WM bundles and neurons of the hippocampus. MMI significantly decreases axon and myelin density, promotes demyelination, impairs synaptic plasticity, and decreases neurite branching and dendritic spine density in the cortex and hippocampus. Water channel dysfunction may contribute to cognitive deficits and WM damage induced by the MMI model.

Disclosures: **P. Venkat:** None. **M. Chopp:** None. **J. Chen:** None.

Poster

743. Learning and Memory: Aging - Other Areas

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Program#/Poster#: 743.11/JJJ40

Topic: H.01. Animal Cognition and Behavior

Title: Characterization of cognition in aging immunocompromised mice

Authors: S. REGE[1], T. HEIKKINEN[2], H. HACKBART[1], *E. CZIRR[1], I. GALLAGER[1], R. HODGSON[2], S. BRAITHWAITE[1], S. MINAMI[1];
[1]Alkahest, San Carlos, CA; [2]Charles River Labs., Kuopio, Finland

Abstract: Assessing cognitive behaviors and their modulation by therapeutics in mouse models is important in the development of treatments for a range of neurodegenerative disorders and other indications associated with cognitive decline. Potential off-target effects generated by experimental therapies could confound behavioral outcomes, which highlights the importance of identifying the appropriate animal model. An example is the testing of human-derived reagents and proteins that could make for attractive therapeutic candidates, but their use in a heterologous species would generate deleterious immune responses. To circumvent this problem immunocompromised mice can be used, however the characterization of their cognitive function as it relates to aging has not been well explored. We studied the effect of aging on two strains of immunocompromised animals, NOD.CB17-*Prkdc*^{scid}/NcrCrl (NOD scid) and NOD.Cg-*Prkdc*^{scid}*Il2rg*^{tm1Wjl}/SzJ (NSG) mice. Animals were compared at 3 months and 12 months of age in assays including open field, Y-maze and contextual fear conditioning. To address variability in performance in behavioral assays between different laboratories, we have conducted studies at two independent sites with independent researchers. Immunocompromised animals show age-related reductions in motor function and cognitive decline within 1 year of age, indicating accelerated aging compared to wild-type animals. These results confirm that immunocompromised animals can be used as a platform to test changes in age-related cognitive processes and other functions, and their modulation by a range of therapeutic agents.

Disclosures: **S. Rege:** A. Employment/Salary (full or part-time): Alkahest. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Alkahest. **T. Heikkinen:** A. Employment/Salary (full or part-time): Charles River Laboratories. **H. Hackbart:** A. Employment/Salary (full or part-time): Alkahest. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Alkahest. **E. Czirr:** A. Employment/Salary (full or part-time): Alkahest. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Alkahest. **I. Gallager:** A. Employment/Salary (full or part-time): Alkahest. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Alkahest. **R. Hodgson:** A. Employment/Salary (full or part-time): Charles River Laboratories. **S. Braithwaite:** A. Employment/Salary (full or part-time): Alkahest. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Alkahest. **S. Minami:** A. Employment/Salary (full or part-time): Alkahest. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Alkahest.

Poster

743. Learning and Memory: Aging - Other Areas

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Topic: H.01. Animal Cognition and Behavior

Support: VA merit review

MH093429

Title: Drug targeting of rev-erb improves cognition in alzheimer's disease mouse model

Authors: ***D. ROBY**[1], F. RUIZ[1], B. KERMATH[1], J. E. MORLEY[2], T. BURRIS[1], S. A. FARR[1]; [1]Pharmacol. and Physiological Sci., [2]Intrnl. Medicine/Geriatrics/Endocrinology, St. Louis Univ., Saint Louis, MO

Abstract: Alzheimer's disease (AD) has a plethora of possible risk factors, but the most certain risk factor is aging. Hallmarks of AD are aggregates of hyper-phosphorylated tau and amyloid-β (Aβ), and impediments in learning and memory are among the most common initial symptoms of the disease. The onset of perturbed sleep patterns suggests that circadian rhythm in AD patients is disrupted, indicating that the central clock is involved in the onset of AD. The central clock is

regulated mainly by nuclear receptors (NR). A key NR, REV-ERB, inhibits and regulates transcription of clock genes and genes involved in long-term potentiation (LTP). REV-ERB's expression is fairly ubiquitous in the brain. Recent studies have shown the correlation between REV-ERB-associated behavior and proteins involved in AD's onset. Aβ accumulation is associated with the disruption of the sleep/wake cycle and impairs the rhythmic fluctuation of Aβ interstitial fluid levels in a transgenic mouse model. Furthermore, Aβ disrupts both the circadian sleep/wake cycle and hippocampal learning formation, suggesting that alterations in REV-ERB expression may be part of Aβ's pathology. Here, we examined the effects of the REV-ERB agonist SR9009 on learning and memory in the SAMP8 mouse model of AD. We studied the effects of SR9009, a specific REV-ERB agonist, in a SAMP8 mouse model. This is an accelerated aging model that exhibits consistent age-related increase in Aβ, hyper-phosphorylated tau, oxidative stress, impaired efflux of Aβ from the brain as well as learning and memory deficits beginning around 8 months and fully developed at 12 months. We treated male SAMP8 mice with SR9009 (100 mg/kg body weight i.p.) beginning at 11 months of age. After 4 weeks of treatment, they exhibited improved learning and memory behavior phenotypes compared to aged SAMP8 mice treated with vehicle. Aged SAMP8 mice treated with SR9009 had learning and memory comparable to that of young SAMP8 mice (4 months) treated with vehicle in novel object recognition and T-maze retention. SR9009 was given post-onset of cognitive deficits, showing that increased REV-ERB activity is able to rescue the cognitive deficits associated with Aβ and tau. This study presents REV-ERB as a potential target in the treatment of AD.

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Poster

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Topic: H.01. Animal Cognition and Behavior

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Campbell Family Mental Health Research Foundation operating grant

Title: Molecular correlates of altered neural communication in ofc layer 2-3 during human aging

Authors: *M. PABBA[1], E. SCIFO[1], F. KAPADIA[1], Y. NIKOLOVA[1], C. MA[2], N. MECHAWAR[3], G. C. TSENG[2], E. SIBILLE[1];

[1]Psychiatry, Pharmacol. and Toxicology, Univ. of Toronto, Campbell Family Mental Hlth. Res. Inst., Toronto, ON, Canada; [2]Univ. of Pittsburgh, Pittsburgh, PA; [3]McGill Univ., Montréal, QC, Canada

Abstract: *Background*: Multidisciplinary studies have demonstrated a decline in orbitofrontal cortex (OFC) cognitive functions, grey matter volume, loss of spines/synapses at layer 3 as well as gene expression changes related to neural communication during the course of normal aging. Recently, the gene expression changes that occur during the course of normal aging have been classified into nine hallmarks of aging based on peripheral tissue. However, the extent to which these hallmarks apply to brain tissue comprised largely of non-dividing cells has not been evaluated. Gene array and functional imaging studies have provided large-scale information on brain aging; however, to date no unbiased high-throughput proteomic studies have been performed on the OFC during human aging. Cortical layers 2/3 have a prominent role in processing feedforward and feedback information within local circuitry and with other regions of the brain. Accordingly, we performed proteomic profiling of the OFC layer 2/3 during human aging. *Prediction*: Proteomic analysis of OFC layer 2/3 during human aging will reveal changes in biological hallmarks of aging, specifically affecting levels of glutamate/GABA/glia-signaling components (i.e., altered neural communication). *Methods and Results*: MS-based proteomics was performed on laser-captured layer 2/3 of postmortem OFC from 15 young and 18 old male subjects. Label free quantification and random-intercept modeling revealed 131 differently expressed proteins (p value ≤ 0.05 and a minimum of 20% effect size), including 66 up- (e.g., GFAP, AQP4) and 65 (e.g., GRM2, 3, CALB1) down-regulated proteins, respectively. Most age-associated proteins were related to altered neural communication. Functional and network analyses on differentially expressed proteins showed enrichment for glutamate-related signaling and processes, for example, learning and memory among many other hallmarks of aging such as deregulated nutrient sensing and loss of proteostasis. Interestingly, age-altered layer 2/3 proteins showed association with many neurological and psychological disorders. *Summary*: Our study identified qualitative and quantitative proteomic changes in the OFC layer 2/3 during the course of aging. Results from this study also confirmed previously identified age-associated molecular changes (hallmarks of aging) from transcriptomic, morphological and imaging studies on the OFC. Changes in neural communication at layer 2/3 may contribute to age-associated decline in OFC related cognitive functions. Overlap between age-altered and disease associated proteins suggests that aging may contribute to acceleration of disease progression.

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Poster

743. Learning and Memory: Aging - Other Areas

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Topic: H.01. Animal Cognition and Behavior

Title: Oral administration of a brain penetrating ghrelin receptor agonist (hm01) inhibits age-dependent memory loss in mice.

Authors: *R. G. SMITH[1], S. P. MAHAL[2];
[1]Metabolism & Aging, Scripps Florida, Jupiter, FL; [2]Metabolism and Aging, Scripps Res. Inst., Jupiter, FL

Abstract: Declining levels of the growth hormone secretagogue/ghrelin receptor (GHSR1a) are associated with age-dependent functional deficits. The lowest levels were found in brains from sporadic Alzheimer's disease patients. Previously we reported that administration of an orally active agonist of GHS-R1a to elderly subjects restored the young adult phenotype.[1] Furthermore, we recently showed in mice that GHSR1a and dopamine receptor-1 (D1R) form heterodimers in hippocampal neurons that are essential for initiation of synaptic plasticity and hippocampal memory.[2] We now report that oral gavage of the GHS-R1a agonist, HMO1, prevents age-dependent memory loss in 10-18 month old mice. HMO1-treated mice show significant improvements in both spatial and contextual memory. In the water maze test remote memory improved significantly (p=0.008) 1 week after acquisition. In fear conditioning, improvements in memory (p=0.011) were detectable (mice at age 18 months), up to 3 months after the initial foot shock.
In summary, our studies show that while aging reduces the concentration of ghrelin receptors in the brain, oral administration of the brain penetrating ghrelin receptor agonist, HM01, augments GHSR1a:DRD1 heteromer signaling and prevents/restores age-related memory loss in mice, most likely via DRD1 mediated hippocampal memory. 1. Smith RG, Sun Y, Jiang H, Albarran-Zeckler R, Timchenko N. Ghrelin receptor (GHS-R1A) agonists show potential as interventive agents during aging. *Ann, N. Y. Acad Sci.* 2007;Nov;(1119):147-164. 2. Kern A, Mavrikaki M, Ullrich C, Albarran-Zeckler R, Brantley AF, Smith RG. Hippocampal Dopamine/DRD1 Signaling Dependent on the Ghrelin Receptor. *Cell.* 2015;Nov 19;163(5):1176-1190. **Theme H: Cognition H.01 Animal Cognition and Behavior** H.01.s Learning and Memory: Aging Nanosymposium prefereed

Disclosures: R.G. Smith: None. **S.P. Mahal:** None.

Poster

743. Learning and Memory: Aging - Other Areas

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 743.15/JJJ44

Topic: H.01. Animal Cognition and Behavior

Support: NIH NIA R01AG037868

NIH Training Grant T32 DK007778

Title: Aged animals appear cognitively and behaviorally hyporesponsive to chronic restraint (psychosocial stress) compared to young animals

Authors: *K. STAGGS, J. POPOVIC, S. QUTUBUDDIN, E. M. BLALOCK;
Pharmacol., Univ. of Kentucky, Lexington, KY

Abstract: It is established that aging has detrimental consequences including a change in sleep architecture, a blunted circadian rhythm, and a decrease in cognition. Psychosocial stress (PS) is a non-painful stimulus associated in humans with major life changes including job loss, death of a spouse, and social isolation. It strongly influences multiple systems (e.g., corticosterone level, body temperature regulation, sleep and cognition). In prior work, we showed that acute PS resulted in typical cognitive deficit and hyperthermia responses in young animals, but that aged animals were hyporesponsive to this acute PS challenge. However, PS in humans is normally chronic, not acute, and the likelihood of experiencing PS increases with age. Nevertheless, little work has investigated the response of chronic PS in aged subjects. We hypothesized that aged animals will continue to be hyporesponsive to chronic PS. To test this, young (3mos) and aged (19mos) male Fischer344 rats were assigned to control or PS groups and implanted with wireless telemetry from Data Sciences International to monitor sleep and body temperature. Chronic PS (restraint, 3 h/day, 4 days/week, 4 weeks) effects on distress response, Morris water maze (MWM), body temperature, and corticosterone levels were collected. Chronic PS did not affect spatial MWM training, deep sleep duration, body temperature, or corticosterone levels at any age. PS resulted in decreased active period wake in aged animals. Conversely, aged animals were hyporesponsive to PS effects on the distress response and MWM probe trial. Taken together, the aged animals appear cognitively and behaviorally hyporesponsive to chronic PS.

Disclosures: K. Staggs: None. **J. Popovic:** None. **S. Qutubuddin:** None. **E.M. Blalock:** None.

Poster

743. Learning and Memory: Aging - Other Areas

Location: Halls B-H

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Topic: H.01. Animal Cognition and Behavior

Support: NIH/NCAT 1TL1 TR001431, Translational Biomedical Science Program, NIH-CTSA funded Georgetown-Howard Universities Center for Clinical and Translational Science

NIH/NHLBI 1R01-HL119380

Title: Effect of ovariectomy on cognitive function in the hypertensive Dahl salt-sensitive rat

Authors: S. P. WRIGHT[1], A. A. DE SOUZA[3,1], A. PAI[1], H. JI[1], X. WU[1], X. TATIN[4], A. S. NARAINE[5], W. HE[5], *R. C. SPETH[6,2], P. A. FORCELLI[2], K. SANDBERG[1];
[1]Dept. of Med., [2]Pharmacol. and Physiol., Georgetown Univ., Washington, DC; [3]Dept. of Biol. Sciences, Inst. of Exact and Biol. Sci., Federal Univ. of Ouro Preto, Ouro Preto, Brazil; [4]AgroParisTech, Paris, France; [5]Biol., Halmos Col. of Natural Sci. and Oceanography, Nova Southeastern Univ., Fort Lauderdale, FL; [6]Dept. of Pharmaceut. Sciences, Col. of Pharm., Nova Southeastern Univ., Davie, FL

Abstract: Previous studies have shown that ovariectomy (OVX) impairs cognitive function in Sprague Dawley rats using the 12-arm radial arm maze. **AIM**: In this study, we investigated if OVX impairs cognitive function in a hypertensive vs. normotensive animal model. **METHODS**: Dahl salt-sensitive (DS) and salt-resistant (DR) female rats underwent OVX or sham-operation (Sham) at 6 weeks of age. Six months later, the animals were evaluated with the Novel Object Recognition Test (NORT). **RESULTS**: Between 8 & 9 months of age, three DS-OVX rats died, presumably due to stroke as a result of the hypertension, as previously we found that OVX accelerated the age-associated increase in arterial pressure in these rats. When we compared the NORT results in the animals that later died with the animals that survived, we found the time spent with the novel object was significantly less [(time spent with novel object / total time spent with novel object and familiar object): DS-OVX-survived, 0.82 ± 0.06 (n=5) vs. DS-OVX-died, 0.60 ± 0.1 (n=3); p = 0.04]. DS-Ovx-survived scores were significantly higher than what would have been expected by chance (p=0.008), but the DS-Ovx-died scores were not, indicating that the DS-Ovx-survived rats recognized the novel object as new, but the DS-Ovx-died rats may not have. Furthermore, the hypertensive DS rats tended to do poorer on the NORT compared to the normotensive DR rats [(time spent with novel object / total time spent with novel object and familiar object): DS, 0.77 ± 0.04 (n=16) vs. DR, 0.85 ± 0.03 (n=15); p = 0.056]. In contrast, no DR rats died and there was no difference detected in the proportion of time spent with the novel object between the DR-OVX and DR-Sham. **CONCLUSION**: This study is consistent with the hypothesis that the hypertension exacerbated by OVX impairs cognitive function in the DS rat.

Disclosures: **S.P. Wright:** None. **A.A. de Souza:** None. **A. Pai:** None. **H. Ji:** None. **X. Wu:** None. **X. Tatin:** None. **A.S. Naraine:** None. **W. He:** None. **R.C. Speth:** None. **P.A. Forcelli:** None. **K. Sandberg:** None.

Poster

743. Learning and Memory: Aging - Other Areas

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 743.17/JJJ46

Topic: H.01. Animal Cognition and Behavior

Support: Protein Folding Diseases Initiative, University of Michigan

Title: The effect of a lifespan-extending drug, acarbose, on cognitive healthspan in a mouse model of Alzheimer's disease

Authors: ***S. J. MOORE**[1], R. C. PARENT[1], R. A. MILLER[2], G. G. MURPHY[3];
[1]Mol. & Behavioral Neurosci Inst., [2]Dept. of Pathology and Geriatrics Ctr., [3]Mol. & Behavioral Neurosci Inst. and Dept. of Mol. and Integrative Physiol., Univ. of Michigan, Ann Arbor, MI

Abstract: As the aged population continues to grow, there is increased interest in discovering and exploiting interventions that can prolong lifespan. The National Institute on Aging's Interventions Testing Program has shown that acarbose, a drug which is already used in the treatment of diabetes because it blunts the post-meal spike in blood sugar, significantly extends lifespan in a genetically heterogeneous mouse line, UM-HET3. However, the question of whether acarbose can also preserve or extend cognitive function in mice as they age has not been explored. In addition, advancing age remains the largest risk factor for developing neurodegenerative diseases like Alzheimer's disease (AD); thus, acarbose treatment may also be effective in preserving or rescuing cognitive function in mouse models of neurodegenerative disease.
To address these questions, we are treating mice with acarbose (delivered ad libitum in their chow) starting at 4 months of age and evaluating the cognitive function of separate cohorts of mice at several different time points throughout the lifespan. Our preliminary data show that in wild-type UM-HET3 mice, even as early as 9 months of age, acarbose improves cognitive function, as assayed by the Morris water maze, compared to mice maintained on control chow. Additionally, in a mouse model of AD (the 5xFAD model, which has five familial AD mutations, including 3 separate mutations in the amyloid precursor protein, APP, and 2 distinct mutations in presenilin-1, PS-1) that has been bred on the UM-HET3 genetic background, acarbose treatment was sufficient to rescue cognitive deficits observed in the Morris water maze at 9 months of age.

Because acarbose is already used in a clinical capacity in humans and is known to be safe and well-tolerated, it has the potential for rapid translation into clinical studies. Together with our results, this suggests that acarbose treatment may be useful in a therapeutic setting to extend lifespan and to improve cognitive outcomes in both healthy aged populations as well as in those impacted by neurodegenerative diseases.

Disclosures: S.J. Moore: None. **R.C. Parent:** None. **R.A. Miller:** None. **G.G. Murphy:** None.

Poster

743. Learning and Memory: Aging - Other Areas

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 743.18/JJJ47

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant MH101491 (MAW)

NIH Grant DA025922 (MAW)

NIH Grant DA036984 (MAW)

NIH T32 Grant AG000096-31 (JLK)

NIH F32 Grant AG052303 (JLK)

Title: The role of HDAC3 in age-related plasticity and memory dysfunction

Authors: *J. L. KWAPIS, Y. ALAGHBAND, E. A. KRAMÁR, A. J. LÓPEZ, A. O. WHITE, D. RHEE, C. M. MICHAEL, G. SHU, D. P. MATHEOS, M. A. WOOD;
Neurobio. and Behavior and Ctr. for the Neurobio. of Learning & Memory, Univ. of California, Irvine, Irvine, CA

Abstract: Aging is accompanied by cognitive impairments, including difficulty forming long-term memories. Long-term memory formation requires gene expression, a process that may be disrupted with age (Rowe et al., 2007; Berchtold et al., 2008). Epigenetic alterations (changes in gene expression that occur through alterations in chromatin structure) may therefore contribute to age-related impairments in gene expression and long-term memory. One major epigenetic mechanism important for memory is histone acetylation, in which acetyl groups are added or removed from histone tails by acetyltransferases (HATs) or histone deacetylases (HDACs), respectively. Increasing histone acetylation by blocking HDAC activity generally enhances both gene expression and long-term memory. In particular, histone deacetylase 3 (HDAC3) appears to

be a key negative regulator of long-term memory formation, as blocking HDAC3 produces persistent object location memory following subthreshold training. Here, we tested whether HDAC3 activity also contributes to age-related impairments in synaptic plasticity and long-term memory. We hypothesized that dysregulated HDAC3 activity in the aging brain contributes to an unusually repressive chromatin structure that limits synaptic plasticity and memory formation. To test this, we disrupted HDAC3 in the dorsal hippocampus of 18-month-old mice with two different manipulations: focal genetic deletion with HDAC3$^{flox/flox}$ mice and activity-specific blockade with a dominant negative point mutant virus (AAV-HDAC3(Y298H)-V5). We found that HDAC3 disruption ameliorated age-related impairments in both long-term memory and synaptic plasticity. To determine the mechanism through which HDAC3 deletion ameliorates memory impairments, we measured gene expression in the dorsal hippocampus of young and aging mice. We found that phosphorylation of HDAC3 was aberrantly increased in the aged brain after learning, even though total HDAC3 levels were similar in young and old brains. We also identified Nr4a1 and Nr4a2 as key downstream mechanisms through which HDAC3 dysfunction may contribute to age-related memory impairments. Together, these results support the hypothesis that HDAC3 is dysregulated with age, leading to age-related impairments in gene expression, long-term memory formation, and synaptic plasticity.

Disclosures: **J.L. Kwapis:** None. **Y. Alaghband:** None. **E.A. Kramár:** None. **A.J. López:** None. **A.O. White:** None. **D. Rhee:** None. **C.M. Michael:** None. **G. Shu:** None. **D.P. Matheos:** None. **M.A. Wood:** None.

Poster

743. Learning and Memory: Aging - Other Areas

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 743.19/JJJ48

Topic: H.01. Animal Cognition and Behavior

Support: Intramural Research Program of the NIA

AG10606

Title: Prefrontal cortex interneuron vulnerability in behaviorally characterized aged monkeys

Authors: K. H. SCHULZE[1], A. M. SPIEGEL[1], *J. M. LONG[1], E. J. PEREZ[1], M. T. ROBERTS[2], P. R. RAPP[1];
[1]Natl. Inst. On Aging-NIH, Baltimore, MD; [2]California Natl. Primate Res. Ctr., Davis, CA

Abstract: Aging is associated with a decline in working memory, a cognitive capacity dependent on area 46 of the dorsolateral prefrontal cortex (PFC) in both humans and animal models. These deficits occur in the absence of dramatic PFC neuron loss, suggesting more subtle changes in circuitry integrity underlie the impairment. The GABAergic network, including parvalbumin immunoreactive (PV-IR) interneurons maintains appropriate network dynamics by regulating activity among excitatory neurons and has been demonstrated to be vulnerable to the negative effects of aging in rodents. The effect of age on PV-IR cell number in the primate PFC, however, is unknown.

Here, PFC PV-IR interneuron number was assessed in 7 young adult (8-13 yrs.) and 15 aged (27-38 yrs.) rhesus monkeys (*Macaca mulatta*) of both sexes. Animals were previously tested on a delayed response test of spatial working memory known to be sensitive to aging and PFC damage. Aged subjects performed poorly relative to a large population of young animals, and comparable to aged monkeys in many previous experiments. For post-mortem histological analysis, animals were perfused with cold fixatives, and the brains were frozen and sectioned in the coronal plane at 40 microns in an evenly spaced 1 in 10 series. For each brain, a single series spanning the rostro-caudal length of area 46 was processed for the immunocytochemical visualization of PV (Sigma, cat#P3088), using diaminobenzidine as the chromagen. Design-based stereological techniques were employed to estimate the total number of PV-IR neurons in both the dorsal and ventral banks of area 46.

The estimated number of PV-IR interneurons in the ventral bank of area 46 was comparable in young and aged monkeys, averaging a total of 639,398 and 559,774, respectively. In contrast, aged monkeys displayed roughly 40% more PV-IR neurons in the dorsal bank relative to young adults (totals = 853,755 vs. 608,609, p=0.05). These results add to a growing body of evidence on interneuron vulnerability in aging, suggesting that disrupted inhibitory circuitry in the PFC is a prominent feature of neurocognitive aging across species.

Disclosures: **K.H. Schulze:** None. **A.M. Spiegel:** None. **J.M. Long:** None. **E.J. Perez:** None. **M.T. Roberts:** None. **P.R. Rapp:** None.

Poster

743. Learning and Memory: Aging - Other Areas

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 743.20/JJJ49

Topic: H.01. Animal Cognition and Behavior

Support: NIA

Title: Quantifying changes in medial prefrontal cortex immediate early gene expression as a function of aging related cognitive decline

Authors: *V. L. EHLERS, M. SEHGAL, B. K. FULLEYLOVE-KRAUSE, J. R. MOYER, Jr; Psychology, Univ. of Wisconsin Milwaukee Dept. of Psychology, Milwaukee, WI

Abstract: The world's population of individuals aged 85 and over is projected to increase by 350% by the year 2050. It is important for research on aging to assess changes in brain function throughout the aging process in order to combat aging-related cognitive decline and to improve the quality of living for the elderly. One way to understand how aging alters the neuronal circuitry that underlies aging-related cognitive decline is to study extinction of fear conditioning. Previous data from our lab suggest that aged rats exhibit impaired fear extinction. Thus the current experiments were designed to assess how immediate early gene (IEG) expression of Zif-268 and c-fos was altered in brain regions implicated in associative learning in order to better understand how overt behavioral deficits might be linked to aging-related changes in the brain. Young, middle-aged, and aged rats were separated into four experimental groups: naïve, pseudo-conditioned (PSEUDO), trace fear conditioned (TRACE), and fear extinction (EXT). On day 1, TRACE and EXT rats underwent trace fear conditioning (10 trials of 15 s tone, 30 s trace interval, 1 s footshock), while PSEUDO rats underwent pseudo conditioning (10 tones and 10 shocks that were explicitly unpaired). On days 2 and 3, EXT and PSEUDO rats were given 10 tone-alone presentations. On day 4, TRACE, EXT, and PSEUDO rats were presented with 2 tone presentations to assess trace fear memory. Brain slices were collected 1-hr after testing to assess IEG expression in various brain regions of interest (e.g., mPFC, hippocampus, amygdala, retrosplenial cortex). Our initial studies did not observe any statistically significant behavioral differences between age groups, however, analysis of mPFC indicated that in adult rats IEG expression was elevated within the prelimbic and infralimbic subregions within the PSEUDO, TRACE, and EXT groups ($p < .05$). Although learning occurred in the aged animals, this learning did not involve significant changes in IEG expression within mPFC, suggesting that aging may result in circuit-level changes in molecular signaling that supports acquisition and extinction of trace fear conditioning.

Disclosures: V.L. Ehlers: None. **M. Sehgal:** None. **B.K. Fulleylove-Krause:** None. **J.R. Moyer:** None.

Poster

744. Human Cognition and Memory V

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 744.01/JJJ50

Topic: H.02. Human Cognition and Behavior

Title: The extreme memory challenge: A search for the heritable foundation of exceptional memory

Authors: ***M. A. PYC**[1], E. GIRON[2], P. CHEUNG[1], S. DE BELLE[1], T. TULLY[1];
[1]Cognitive Sci., Dart NeuroScience LLC, San Diego, CA; [2]Dart NeuroScience, San Diego, CA

Abstract: We are interested in discovering new candidate targets for drug therapies to enhance cognitive vitality in humans throughout life, and to remediate memory deficits associated with brain injury and brain-related diseases such as Alzheimer's and Parkinson's. To achieve our goal we need a comprehensive and objective understanding of the human genome contribution to variation in memory performance in healthy individuals. We are implementing a Genome-Wide Association Study (GWAS) to identify genetic loci varying among individuals who possess exceptional and normal memory abilities. These genes and those in associated networks will inform drug discovery and development. Our first step is to identify exceptional members of the population. Thus, we have created an online memory test - the Extreme Memory Challenge (EMC) - to conveniently screen through an unlimited number of subjects to find individuals with exceptional memory consolidation abilities. Identified subjects are (1) validated by a battery of secondary memory tasks, and (2) providing saliva samples from which we can isolate DNA for GWAS. Ten pilot experiments were conducted to parameterize the EMC screen. Participants learned face-name pairs for a delayed recall test. After initial study, each name was presented and participants were asked to select the correct face among four (distracters were other faces paired with different names). One day later participants completed a final test trial. We are primarily interested in forgetting across sessions, as this provides an estimate of consolidation across a 24-hour time interval. Pilot studies indicated the optimal protocol should include 30 face-name pairs, presented at a 4 second rate. To date, 14,010 participants from 173 nations have been screened in the EMC. Of these, 9,189 have completed both sessions. Individuals in our sample are most frequently Caucasians (56%), US citizens (40%), native English language speakers (45%), post-secondary school-educated (63%), reported being most alert in the morning (52%), with good vision (70%), and right handed (89%). The gender distribution was split evenly. Performance in the EMC decreased across sessions by 8% (from 60% to 52%). We identified 22 subjects scoring 3+ standard deviations from the forgetting mean, and 23 scoring perfectly during both sessions. For both of these exceptional groups, performance is being validated by our memory battery prior to the genomics portion of the project, now underway.

Disclosures: **M.A. Pyc:** None. **E. Giron:** None. **P. Cheung:** None. **S. de Belle:** None. **T. Tully:** None.

Poster

744. Human Cognition and Memory V

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 744.02/JJJ51

Topic: H.02. Human Cognition and Behavior

Title: Stressed management women increase memory task efficiency after a 8-week mindfulness-based positive emotions cultivating program

Authors: *D. F. SANTAELLA[1], J. B. BALARDIN[1], B. PORTES[1], P. TOBO[2], C. BARRICHELLO[2], S. S. LACERDA[1], J. R. SATO[3], E. AMARO JR.[1], E. H. KOZASA[1]; [1]Brain Inst., Hosp. Israelita Albert Einstein, Sao Paulo, Brazil; [2]Natura Inovação, São Paulo, Brazil; [3]Univ. Federal do ABC, São Paulo, Brazil

Abstract: Default mode network (DMN) is a connectivity system involved in different resting state functions, such as autobiographical information; self-referenced memories; referring to descriptions and emotions of one's self; moral reasoning; social evaluations; thinking about other one's thoughts; remembering the past, imagining the future and episodic memory, present during mind wandering processes. Conectivity studies have demonstrated that DMN and working memory may not have opposite functions and that DMN and WMN (working memory network) may both be active during a memory task. Corporative environments have a great stress load on managers, specially women (with double shifts, facing home duties) and are prone to burnout. Objective: To investigate the effects of a 8-week mindfulness-based program of cultivating positive emotions, ethics and healthy interpersonal attitudes (Florescer) on brain functional activation during a memory task (n-back) in stressed management women. Methods: sixty workers from Natura Cosmetics S.A. were invited; 59 were included, 24 finished the 8-week program. After giving written consent, participants were evaluated for: class presence, home practice, age, and were submitted both to a resting state and to a memory task (N-Back) inside a functional magnetic resonance 3T device. During the memory task, subjects were instructed whether to find a specific letter, or to remember if it had been presented one or two positions behind. Significance was set at $p<0.05$, with $Z>2.3$. Data was processed using FSL software. Results: Both groups were equal at study entry for correct responses and reaction time in the "find" task: Florescer vs. Control (correct responses 6.9±2.8 vs. 6.6±2.6 p=0.963 and reaction time 591±113 vs. 678±145 ms p=0.405). Florescer program did not change these parameters and groups remained equal at 8 weeks: (7.7±1.3 vs. 7.1±2.1 p=0.350 and 580±97 vs. 598±93 ms p=0.783). Regarding functional activations, groups demonstrated different activation patterns when submitted to the memory task after training: Florescer increased and Control decreased activation on DMN structures such as: posterior cingulate gyrus, precuneus, hippocampus, frontal medial cortex, posterior cingulate gyrus, frontal pole, anterior cingulate gyrus, lingual gyrus; besides those, corpus callosum also showed greater activation pattern. Conclusion:

Florescer program increased DMN engagement during a memory task in corporative environment distressed women, possibly indicating that this program allowed participants to perform the task in a more relaxed inner attitude; a smaller attentional engagement and with increased efficiency.

Disclosures: **D.F. Santaella:** None. **J.B. Balardin:** None. **B. Portes:** None. **P. Tobo:** None. **C. Barrichello:** None. **S.S. Lacerda:** None. **J.R. Sato:** None. **E. Amaro Jr.:** None. **E.H. Kozasa:** None.

Poster

744. Human Cognition and Memory V

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 744.03/JJJ52

Topic: H.02. Human Cognition and Behavior

Support: DFG Grant HA 5622/1-1

Title: Is transcranial alternating current stimulation an effective tool for cognitive research?

Authors: *V. BRAUN, S. HANSLMAYR;
Sch. of Psychology, Univ. of Birmingham, Birmingham, United Kingdom

Abstract: Transcranial alternating current stimulation (tACS) is widely used to entrain or modulate brain oscillations and shed light onto the relationship between oscillatory activity and cognitive processes. tACS could be an effective tool for cognitive research if it was able to modulate brain oscillations in a time critical manner. During cognitive tasks, brain oscillations show a highly dynamic behaviour. For instance beta oscillations decrease in power within a couple of milliseconds during memory processing followed by a subsequent increase in amplitude. If tACS should be useful for asking causal questions about these dynamics it must influence brain oscillatory behaviour in a similar time range. In a series of experiments we addressed the question whether event-related, transient tACS in the beta frequency range can be used to modulate 2 different processes: episodic memory formation and motor cortex excitability. Experiments 1 and 2 aimed at replicating and extending findings from a recently published TMS study. By using rTMS it has been shown that entrainment of left prefrontal beta oscillations leads to impaired memory encoding of verbal material. In order to replicate and extend these findings, 72 healthy human participants engaged in an incidental encoding task of verbal and non-verbal material while receiving tACS to the left and right inferior frontal gyrus (IFG) at 6.8Hz, 10.7Hz, 18.5Hz, 30Hz, 48Hz and sham stimulation for 2s during stimulus presentation. Experiment 3 addressed the question whether 10s of beta tACS is sufficient to

entrain brain oscillations in the primary motor cortex (M1). By administering tACS to M1 at the individual motor beta frequency of 8 subjects, we investigated the relationship between the size of TMS induced MEPs and tACS phase. The present results indicate that applying tACS over a short period of time (2s; 10s) did not entrain brain oscillations and did not affect behaviour. In experiments 1 and 2, findings from a TMS study could not be replicated. Beta tACS did not affect memory performance. Likewise, no entrainment effects could be found in experiment 3. MEP size was not modulated by the tACS phase, indicating that our stimulation protocol did not entrain beta oscillations in M1. Taken together these findings suggest that using tACS in an event-related manner and applying the stimulation for short periods of time is not effective in modulating brain oscillatory activity. These failures of event-related tACS to modulate cognition or cortical excitability cast doubt on whether tACS could be an alternative to rTMS in unravelling the causal relationship between brain oscillatory activity and cognitive processes.

Disclosures: V. Braun: None. **S. Hanslmayr:** None.

Poster

744. Human Cognition and Memory V

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 744.04/JJJ53

Topic: H.02. Human Cognition and Behavior

Title: Environmental encoding and brain structure

Authors: *H. R. EVENSMOEN, L. RIMOL, T. HANSEN, A. HÅBERG;
NTNU, Trondheim, Norway

Abstract: The relationship between encoding of the different associations that our environmental representations consist of and cortical area and thickness, and the size of different structures in the brain, is to a large extent unknown. In this study, the participants had to learn either 20 or 35 small virtual environments. The environments consisted of five objects that formed a unique positional pattern within the environments. Each environment was enclosed by one of 10 differently shaped outer walls. The participants had to complete several runs. Each run involved five environments. After each run, the participants' ability to associate the objects together, associate the objects with the outer wall, recreate the objects positional pattern, place the objects positional pattern relative to the outer wall, place the objects in their position within the positional pattern, were tested for each environment. The association between the different environmental measures and cortical thickness and area, and subcortical volumes, were investigated in Freesurfer. The relationship between the environmental measures and the size of

the different subregions along the anterior-posterior axis of the hippocampus were also investigated, using a high resolution T2 image and ASHS.

Disclosures: **H.R. Evensmoen:** None. **L. Rimol:** None. **T. Hansen:** None. **A. Håberg:** None.

Poster

744. Human Cognition and Memory V

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 744.05/JJJ54

Topic: H.02. Human Cognition and Behavior

Support: NIMH Grant R01-MH07458

Title: Comparison of the ERP correlates of encoding item-item and item-context associations

Authors: ***J. X. WONG**, J. D. KOEN, L. J. LEWIS, M. D. RUGG;
Ctr. for Vital Longevity, Univ. of Texas At Dallas, Dallas, TX

Abstract: A key feature in forming episodic memories is the establishment of associations between different components that comprise the memory episode. In order to investigate the neural correlates underlying memory formation, we can contrast neural activity during study for items that are remembered or forgotten on a subsequent memory test, with the assumption that the processes indexed by this contrast contribute to whether an item subsequently receives an accurate memory judgment. In a previous fMRI study, we had participants perform an incidental encoding task where they studied both item-item ('associative') and item-context ('source') information in each trial. We found that successful encoding of associative information was accompanied by 'subsequent memory effects' in cortical brain regions distinct from those for successful source encoding. Here, we employed EEG/ERP to the study/test procedure used in our fMRI study in order to characterize the temporal correlates of encoding the same two types of memory associations. We recorded EEG during the incidental encoding task, where participants studied both associative (picture-word pair) and source information (left/right picture location). At test (not recorded), participants saw both studied and new pictures, and the requirement was always to judge whether a picture was old/new, and then to judge either its studied location (left/right), or its studied associate (out of two previously studied words). We formed ERPs separately for study trials later tested for location (source) and associative memory, and further segregated these according to the accuracy of the respective memory judgments. We found a polarity reversal in the subsequent memory ERPs, in which more positive-going ERPs were found for successful associative encoding, whereas more negative-going ERPs were found for successful encoding of source information. This polarity reversal suggests that successful

associative and source memory encoding are supported by qualitatively distinct encoding operations. These findings correspond with prior ERP studies that found greater positivity for encoding semantic information, and greater negativity for encoding non-semantic information. We also found a temporal dissociation, in which the more negative-going source subsequent memory effects emerged at picture onset, and were sustained until word presentation, whereas the more positive-going associative subsequent memory effects emerged only after word onset. The current data are consistent with our prior fMRI findings that distinct encoding processes contribute to the subsequent memorability of different types of associations.

Disclosures: **J.X. Wong:** None. **J.D. Koen:** None. **L.J. Lewis:** None. **M.D. Rugg:** None.

Poster

744. Human Cognition and Memory V

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 744.06/JJJ55

Topic: H.02. Human Cognition and Behavior

Title: Consolidation modulates learning following prediction errors

Authors: ***O. BEIN**, L. DAVACHI;
Psychology, New York Univ., New York, NY

Abstract: Prediction errors (PEs) are thought to play a critical role in learning. Recent work has focused on the role of the hippocampus in the detection of memory-based PEs. However, our understanding of how new learning and previous memories are affected by prediction violation is scarce. Furthermore, it is unclear how memory PEs might differ and invoke different kinds of learning for old compared to newly acquired prior memories. Prominent theoretical frameworks propose that PEs should lead to the initial creation of a separated memory trace in the hippocampus (Love, Medin and Gureckis, 2004; McClelland, McNaughton and O'Reilly, 1995) that is later integrated into our knowledge stores. However, this may differ for old and new memories. More consolidated memories may be represented in cortex and thus this may reduce interference within newly learning hippocampal associations. Thus to examine learning following PEs, we presented participants with a stream of objects. Unbeknownst to the participants, the stream was composed of repeating sequences of objects (A-B-C), as well as single objects (D) interspersed between the triads. After initial learning, we induced PEs by presenting the stream of objects again, but replacing the last object in each sequential triad (C-items) with another equally familiar objects (D-items). Critically, for half of the triads, the initial learning occurred immediately before the PE induction, while for the other half, learning occurred the previous day, thus allowing for some consolidation of learned sequences. Memory

integration was assessed by an associative priming test. Our initial results show evidence for integration of old C items with new PE D items, however this was only seen for the more consolidated triads. Specifically, we see that D-items primed the C-items of the initially learned triads, only when prior knowledge was consolidated, but not when it was recent. The results suggest that consolidation may promote new learning by facilitating the integration of novel associations into knowledge. Preliminary imaging analysis will focus on the role of the medial temporal lobe in mediating learning following prediction errors.

Disclosures: O. Bein: None. **L. Davachi:** None.

Poster

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Program#/Poster#: 744.07/JJJ56

Topic: H.02. Human Cognition and Behavior

Title: An Investigation of tDCS-induced modulations of episodic memory encoding

Authors: *G. GALLI, V. NEACSU, F. J. NEWTON;
Dept. of Psychology, Kingston Univ., Kingston Upon Thames, United Kingdom

Abstract: A typical observation in memory research is that items learned using semantic or "deep" operations, such as attending to the meaning of the item, are better remembered than items encoded using "shallow" operations, such as attending to its structural features. Neuroimaging and brain stimulation studies have consistently demonstrated that effective deep encoding is associated with the engagement of the left ventrolateral prefrontal cortex (VLPFC). It is not clear however whether effective shallow encoding engages the same brain region, or more posterior areas such as the parietal cortex. Whether or not episodic encoding relies on a single neural system irrespective of encoding task is therefore still unknown. In the present study we attempted to address this question using anodal transcranial direct current stimulation (tDCS). Thirty-two participants received anodal tDCS while they encoded lists of words using deep and shallow encoding strategies. Half of the participants received the stimulation over the left VLPFC, the other half over the left parietal cortex. Memory for the words was then assessed with a recognition memory test. As typically observed, we showed that items encoded with a deep encoding strategy were better remembered than items encoded using a shallow encoding strategy. This effect however was not modulated by anodal tDCS in either group. Overall, anodal stimulation did not induce any significant change in memory performance compared to sham. These results contribute to the existing debate regarding the effectiveness of electrical current stimulation in modulating higher cognitive functions by showing a lack of behavioural effects of

tDCS, at least in the context of the present stimulation protocol and memory task. Further studies are needed to understand under which condition, if any, tDSC induces changes in memory abilities.

Disclosures: **G. Galli:** None. **V. Neacsu:** None. **F.J. Newton:** None.

Poster

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Program#/Poster#: 744.08/JJJ57

Topic: H.02. Human Cognition and Behavior

Support: Cornell Startup Fund

Title: Dynamic default network engagement as a function of stimulus familiarity and task performance

Authors: ***J. LAM**[1], G. TURNER[1], N. SPRENG[2];
[1]Psychology, York Univ., North York, ON, Canada; [2]Human Ecology, Cornell Univ., Ithaca, NY

Abstract: Numerous studies have demonstrated that default network activation precedes and predicts subsequent errors in goal-directed tasks. A consistent attribute of these tasks is that the stimuli are devoid of personal knowledge or significance. Recently, we devised an N-back task for faces, which included both unknown and famous individuals. Famous faces spontaneously elicited default network activity, which persisted through a 2-back match interval, and was associated with better task performance. Here we extend this line of research by examining dynamics of default network activity during successful and unsuccessful trials on a 3-back working memory task with famous faces as targets (with intervening anonymous face distractors) or distractors (during anonymous face target trials). Young adult participants (N = 20) underwent fMRI scanning while completing a 3-back working memory task with two core conditions: Fame relevant/Anonymous irrelevant (FAAF) and Anonymous relevant/Fame irrelevant (AFFA). This study served to identify dynamic default network engagement as a function of stimulus familiarity and task performance. Consistent with prediction, successful FAAF performance was associated with activation of the default network (posterior cingulate cortex (PCC), anterior and lateral temporal lobes, ventromedial prefrontal cortex, and angular gyrus) and attentional control (inferior and superior parietal lobule, and insula), and visual association areas (fusiform face area). Successful AFFA trials were associated with a pattern of activity including attentional control (superior and inferior parietal lobule, frontal eye fields, insula and MT+) and visual association brain regions (fusiform face area). In contrast, unsuccessful AFFA trials were

associated with co-activation of default regions (hippocampus, angular gyrus, ventromedial prefrontal cortex, PCC) and attentional control areas brain regions (dACC, insula). FAAF errors were associated with a more circumscribed pattern of default and attentional control network activity (PCC, inferior parietal lobule right dorsolateral prefrontal cortex, frontal eye fields, insula). These data provide strong support for the dynamic role of the default network in goal-directed processing. Flexible activation of default network brain regions with attentional control areas and higher order perceptual brain regions can both support or disrupt external goal-direct processing, depending on the task relevance of stored representations.

Disclosures: J. Lam: None. **G. Turner:** None. **N. Spreng:** None.

Poster

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Program#/Poster#: 744.09/JJJ58

Topic: H.02. Human Cognition and Behavior

Support: DARPA N66001-14-C-4016

WFBMC Dept. of Neurosurgery

Title: Human hippocampal recording and stimulation: Validation of depth electrode placement through combined high-resolution imaging and electrophysiologic recordings

Authors: R. WICKS[1], M. WITCHER[1], D. COUTURE[1], A. LAXTON[1], G. POPLI[1], M. SOLLMAN[1], D. SONG[2], V. MARMARELIS[2], T. BERGER[2], S. DEADWYLER[1], *R. E. HAMPSON[1];
[1]Wake Forest Sch. of Med., Winston Salem, NC; [2]USC, Los Angeles, CA

Abstract: Accurate localization of depth electrodes within the CA3 and CA1 subfields in the human hippocampus is critical for the detailed analysis of the neuronal circuitry and potential future applications in memory restoration. To date clinical studies of hippocampal function in humans has been based on imaging with depth electrode localization, a method limited for functional anatomic resolution. Likewise, electrophysiological recordings in human hippocampus have had non-precise anatomic localization.
Eleven adult human patients with medically refractory epilepsy underwent implantation of multiple, stereotaxically-placed, electrodes for seizure localization. Each patient had at least one multisite cellular-recording electrode placed within the largest (head) region of hippocampus which was confirmed using post-operative, high-resolution 3T MRI. Cognitive tasks were then

administered to 8 patients, consisting of sample image presentation within a visual Delayed-Match-to-Sample (DMS) paradigm. Accuracy of electrode placement within the CA3 and CA1 subfields of these patients was assessed via analyses of single unit neuronal firing patterns of 'pairwise' CA3-to-CA1 correlograms during sample and match response phases of the DMS task.
Comparison of pre- and post-operative MRI confirmed that at least one multi-site electrode in each patient was localized with recording sites positioned in both the CA1 and CA3 subfields. Electrode locations that were putatively identified (on the basis of probe morphology and post-operative MRI) as CA3 vs. CA1 were confirmed to exhibit electrophysiological correlates of the well-performed DMS task. Pairwise cross-correlations between putative CA3/CA1 cell pairs revealed that 16% exhibited feedforward activation, while 64% exhibited simultaneous activation consistent with synchronous activation of the CA3 and CA1 cell layers via perforant path during the DMS task. Only 3% of cell pairs exhibited negative cross correlation lag and feedback activation associated with mis-identification of either the CA3 or CA1 neuron within the pairs. Nonlinear modeling of CA3-to-CA1 encoding confirmed the functional connectivity during the task and will be used to validate behavioral relevance during testing of a related neural prosthetic designed to facilitate patient memory within the task.

Disclosures: **R. Wicks:** None. **M. Witcher:** None. **D. Couture:** None. **A. Laxton:** None. **G. Popli:** None. **M. Sollman:** None. **D. Song:** None. **V. Marmarelis:** None. **T. Berger:** None. **S. Deadwyler:** None. **R.E. Hampson:** None.

Poster

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NSF Graduate Research Fellowship Program

Title: Differential responsivity of left and right rostrolateral prefrontal cortex to temporal order violations during the retrieval of real world memories

Authors: *T. E. CHOW, A. J. WESTPHAL, J. RISSMAN;
Dept. of Psychology, UCLA, Los Angeles, CA

Abstract: Rostrolateral prefrontal cortex (RLPFC) critically supports a range of higher cognitive processes, including the control and monitoring of episodic retrieval. Here we aimed to better characterize the contribution of distinct left and right lateralized RLPFC regions during the retrieval of contextually-rich real world autobiographical memories. Using short sequences of photographs as probe stimuli, we examined the degree to which RLPFC activity was modulated as a function of whether the photos of the event were novel or familiar and whether the temporal sequence of the event unfolded in the proper order. Eighteen subjects wore necklace-mounted digital cameras to photograph their daily lives over the course of 3 consecutive weeks. A total of 120 photographic sequences, each consisting of 8 unique images, were selected to represent distinctive events in participants' lives. Participants underwent fMRI scanning while making mnemonic judgments about these events, half of which had been previously encountered during a laboratory session that occurred the day before. Furthermore, half of all events in the fMRI scan session were also presented in a temporally scrambled manner, where the last 4 images within a photo sequence were shown in a different order than what originally occurred. Brain activity was interrogated as a function of photo pre-exposure status (previewed vs. non-previewed) and temporal order (intact vs. scrambled). Our analyses demonstrated a striking dissociation in the activation profile of left and right hemisphere RLPFC regions. Left RLPFC showed greater activity for temporally scrambled photo sequences relative to intact event sequences, but only when the photo sequences were being experienced for the first time. Since temporal order violations in such sequences could not be detected based on comparison with one's memory-derived expectations, this result suggests that left RLPFC may play a role in evaluating whether each successive photo is consistent with one's schema for how events of the type depicted tend to unfold. Interestingly, right RLPFC also showed greater activation for scrambled than intact photo sequences, but only when the sequences had been pre-exposed. This suggests a role in monitoring for a memory-based prediction error (i.e., detecting when the photo sequence was inconsistent with the way it was experienced the prior day). Taken together, this dissociation provides evidence that left and right RLPFC may be sensitive to distinct facets of temporal order violations and may contribute in different ways during autobiographical memory retrieval.

Disclosures: T.E. Chow: None. **A.J. Westphal:** None. **J. Rissman:** None.

Poster

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Topic: H.02. Human Cognition and Behavior

Support: DST Grant DST/SR/2001/S-VYASA/15

Title: Neural correlates of working memory following the practice of meditation - a high-density EEG study

Authors: *S. DEEPESHWAR[1], S. VINCHURKAR[2], K. NAVEEN[2];
[1]Swami Vivekananda Yoga Res. Fndn., Bangalore, India; [2]Swami Vivekananda Yoga Anusandhana Samsthana (SVYASA), Bangalore, India

Abstract: Background: Meditation has been described as training in awareness, which produces definite changes in perception, attention and cognition. This study aimed at mapping the neural correlates of working memory before and after the practice of meditation.
Materials and Methods: Fifty healthy male participants with age ranging from 18 to 35 years were recruited for this study (mean age ± SD was 27.1 ± 5.8 years). Participants with at least one year of meditation experience were enrolled for the study. This study was conducted at a residential yoga university where subjects were assessed in a self as control pre-post design and were randomly tested for meditation (Cyclic meditation) and control sessions (supine rest) on two separate days.
Electroencephalography (EEG) was recorded using a standard 128 Channel EEG system while performing a Visual N back task. Event related potentials (ERP) were derived and source estimates were mapped using standard protocols. Accuracy of the responses and reaction times were included for analysis.
Results: Reaction times were significantly lower and accuracy on the task was significantly higher following the practice of meditation. Subjects exhibited shorter latencies and significantly higher N100 and P300 amplitudes following the practice of meditation. sLORETA constraint was used for source estimation. The regions of highest activation were Fusiform gyrus, parahippocampal gyrus, middle occipital gyrus and the Uncus.
Conclusion: These findings suggest the role of meditation practice in facilitating sustained attention, visuo-spatial processing and executive functions.
Keywords: Meditation, Visual N back, N100, P300, working memory

Disclosures: **S. Deepeshwar:** A. Employment/Salary (full or part-time): Full, Swami Vivekananda Yoga Anusandhana Samsthana (S-VYASA). **S. Vinchurkar:** None. **K. Naveen:** None.

Poster

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Topic: H.02. Human Cognition and Behavior

Support: DNRF93

R01 MH066079

Title: Predicting involuntary memories

Authors: ***S. A. HALL**[1], K. H. ZHU[1], K. E. BRODAR[2], M. HONG[3], P. A. KRAGEL[4], D. LI[1], D. BERNTSEN[5], D. C. RUBIN[1];
[1]Psychology and Neurosci., Duke Univ., Durham, NC; [2]Publ. Hlth., Univ. of North Carolina, Chapel Hill, Chapel Hill, NC; [3]Psychological Sci., Vanderbilt Univ., Nashville, TN; [4]Inst. of Cognitive Sci., Univ. of Colorado, Boulder, Boulder, CO; [5]Dept. of Psychology and Behavioural Sci., Aarhus Univ., Aarhus, Denmark

Abstract: Memories that arise without retrieval effort, or involuntary memories, are an important and frequent form of autobiographical memory and when disrupted, can be an important symptom of mental disorders like post-traumatic stress disorder. Previous work has shown increased activity in the medial temporal lobes and frontoparietal regions during the encoding of items that are later remembered voluntarily compared to those that are not. These effects are referred to as difference due to memory effects (DM). Further, it has been shown that during the encoding of emotionally negative film clips, there is increased activity in regions typically associated with emotion processing, like the amygdala and the rostral anterior cingulate cortex, during the viewing of scenes that are later recalled involuntarily compared to those that are not. However, the majority of involuntary memories experienced in people without mental disorders are emotionally neutral or even positive. An analyses of DM effects that predict the occurrence of involuntary memories across a range of emotional valence levels would allow us to predict these vital memories. To do this, during encoding in the MR scanner, sounds were paired with pictures from the IAPS dataset with emotional valence ratings ranging from neutral to negative. During retrieval in the MR scanner, participants heard the sounds and did an unrelated sound laterality judgment. After the retrieval scan, participants heard the sounds and determined whether its associated picture had been involuntarily recalled during the retrieval scan. As a control condition, unpaired sounds were also presented during encoding and retrieval. Only paired sounds that elicited an involuntary memory during the retrieval scan and unpaired sounds that did not elicit a memory were analyzed. A whole-brain searchlight MVPA was performed on the encoding data. A classifier was trained to differentiate between remembered paired sounds and not-remembered unpaired sounds. The classifier correctly differentiated

between remembered and not remembered trials 65% of the time. Pattern differences between these two conditions were in the supramarginal gyrus, thalamus, insula, caudate, posterior cingulate cortices, bilateral somatosensory cortex, and cerebellum. The involvement of the supramarginal gyrus, thalamus, and insula is consistent with previous work that has shown DM effects in these regions for voluntary memories. Differences in these regions may suggest that items that are associated with more elaborative encoding are more likely to be remembered involuntarily than those that are not.

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Poster

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Topic: H.02. Human Cognition and Behavior

Support: Marie Skłodowska-Curie Actions (H2020- MSCA-IF-2014)

Title: Saccadic eye movements are phase-locked to posterior alpha oscillations during successful memory formation - evidence from MEG, fMRI and intracranial data

Authors: *T. STAUDIGL[1], E. HARTL[2], S. NOACHTAR[2], I. C. WAGNER[1,3], C. F. DOELLER[1], O. JENSEN[1];
[1]Donders Ctr. for Cognitive Neuroimaging, Radboud Univ., Nijmegen, Netherlands; [2]Epilepsy Center, Dept. of Neurol., Univ. of Munich, Munich, Germany; [3]Donders Inst. for Brain, Cognition and Behaviour, Radboud Univ. Nijmegen Med. Ctr., Nijmegen, Netherlands

Abstract: The sampling of visual information is assumed to be discrete rather than continuous (VanRullen & Koch, 2003). Empirical work suggests that the visual system samples the environment at 7-12 Hz, possibly clocked by alpha oscillations (VanRullen et al., 2011). This relatively slow sampling period at 80-140 ms seems at odds with the remarkably fast processing speed of the visual system. For instance, it has been demonstrated that the visual system can distill meaning from images presented for only 13 ms (Potter et al., 2014). This conundrum could partly be resolved if saccades are locked to the phase of ongoing visual oscillations, as investigated in this study.
We simultaneously recorded MEG and eye tracking data from 36 healthy participants during a free viewing encoding task of natural pictures. Memory was subsequently tested in order to classify the encoding trials as ‘later remembered’ versus ‘later forgotten’. MEG data were

aligned to the onset of saccades. Analyses of inter-trial coherence revealed significantly higher phase-locking in the alpha (8-12 Hz) band prior to saccades for later remembered vs. later forgotten pictures. The source of this effect was localized to the parieto-occipital cortex. Intracranial data recorded directly from the occipital and parietal cortex of epilepsy patients provided converging results. Additionally, fMRI data was collected to investigate saccade-related, hippocampal activation and connectivity with the parieto-occipital cortex during successful memory formation.
The study provides evidence that saccadic eye-movements and brain oscillations are coordinated, and demonstrates that this coordination determines what the brain encodes. Specifically, our results suggest that saccades are timed to the dynamic state of the brain, such that the new retinal inputs are temporally aligned to the 'optimal' phase of the alpha rhythm. Concurrent connectivity analyses of source-level MEG and fMRI data will provide first insights into the communication between the visual system and the hippocampus during memory formation, and how this communication is modulated by saccadic eye movements.

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Poster

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Support: JSPS NEXT program LZ001

JSPS KAKENHI 16K04423

Naito Foundation

Title: Effects of aging on activations in the dorsomedial prefrontal cortex and hippocampus during encoding with autobiographical reasoning

Authors: ***T. TSUKIURA**[1], K. NORIMOTO[1], R. YAMAWAKI[1,2,3], Y. SHIGEMUNE[1,4]; [1]Cognitive & Behavioral Sci., [2]Human Hlth. Sci., Kyoto Univ., Kyoto, Japan; [3]Rehabil. Unit, Kyoto Univ. Hosp., Kyoto, Japan; [4]Cognitive Neurol. and Dementia Res., Otto-von-Guericke Univ. Magdeburg, Magdeburg, Germany

Abstract: Autobiographical reasoning is important in constructing stories about the self and one's life. Previous studies have demonstrated that the self-referential processing such as

autobiographical reasoning enhances the memory encoding (SRE: self-reference effect). However, little is known about the age-dependent changes of neural mechanisms underlying SRE by autobiographical reasoning. The current fMRI study investigated this issue. In this study, we employed 25 healthy young (10 females, mean age: 21.4) and 22 healthy older adults (12 females, mean age: 65.8). During encoding with fMRI, participants were presented with pictures selected from IAPS, and were required to view them in two conditions. One condition was the self-related reasoning (Self), in which participants viewed pictures by projecting themselves onto the pictures, whereas in the other condition, participants viewed target pictures by a third person's view such as TV shows or newspapers (Other: other-related reasoning). During retrieval without fMRI, participants recognized whether the pictures were old or new with high and low confidence. All encoding trials were categorized into Hit including subsequent hits with high confidence, and Miss including subsequent hits with low confidence and misses with high and low confidence in each of Self and Other. In behavioral data, both young and older adults remembered targets more accurately in Self than in Other, but the enhancing effect was larger in young adults than in older adults. In fMRI data, successful encoding activations (Hit vs. Miss) in the hippocampus were significantly decreased by the effect of aging. In addition, the dorsomedial prefrontal cortices (dmPFC) showed age-related decrease of activations in Self-Hit vs. Other-Hit, but no age-related difference of dmPFC activations was found in Self-Miss vs. Other-Miss. In the gPPI analyses for young adults, functional connectivity between the dmPFC and hippocampus in Self-Hit was correlated with individual scores of Hit rates, whereas older adults did not show the correlation. These findings suggest that SRE by autobiographical reasoning could be reduced by aging, and the age-related declines of SRE could reflect lower functional connectivity between the dmPFC and hippocampus in older adults.

Disclosures: **T. Tsukiura:** None. **K. Norimoto:** None. **R. Yamawaki:** None. **Y. Shigemune:** None.

Poster

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Topic: H.02. Human Cognition and Behavior

Support: NDSEG Fellowship

Title: Serial dependence in spatial working memory: attraction not swaps

Authors: *D. BLISS, M. D'ESPOSITO;
Helen Wills Neurosci. Inst., UC Berkeley, Berkeley, CA

Abstract: Since 2009, the swap model has been the prevailing account of inter-item interference in continuous-report tests of working memory. A "swap" is defined as a misbinding of item features that causes a subject to report the feature value for the wrong item when probed. An alternative account proposes that instead of inducing swaps, non-target items exert a more subtle pull of the target feature value in the direction of the non-target value, with a magnitude dependent on the distance between the target and non-target values. This effect has been termed "attraction." For the first time in humans, we find evidence of a serial dependence in responses on a test of spatial working memory (the classic oculomotor delayed-response task), consistent with the attraction account. Specifically, the response on each trial is pulled a few degrees in the direction of the previous trial's stimulus (now a non-target) as a function of the distance between the two stimuli. This serial dependence is clearly present in each of our three subjects. The magnitude of the effect grows with memory delay up to about six seconds. We develop a novel formal model to account for the attraction effect and demonstrate that it provides a far better fit to the data than the swap model. Furthermore, the attraction model fully accounts for what has been considered the smoking gun of the swap phenomenon: a central tendency in the deviation of responses from non-target values. As the second stage of our modeling effort, we review evidence that speaks to the neural basis of the identified attractive serial dependence, and formulate a framework for moving from an abstract mathematical model to a concrete neural one.

Disclosures: **D. Bliss:** None. **M. D'Esposito:** None.

Poster

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Topic: H.02. Human Cognition and Behavior

Support: The John Templeton Foundation, Grant # 36751

The John Templeton Foundation, Grant # 57876

Intel Corporation

Title: Blast from the past: Episodic memory supports working memory maintenance

Authors: ***A. NOVICK**, A. M. BORNSTEIN, K. A. NORMAN, J. D. COHEN;
Princeton Neurosci. Inst., Princeton Univ., Princeton, NJ

Abstract: Humans rely on multiple types of memory to make decisions. In particular, when an item must be remembered, it can be committed to working memory (WM) for active maintenance or episodic memory (EM) for later retrieval. Traditionally these have been considered in isolation of one another. For example, short term retention in the absence of distraction is often assumed to rely on WM function. However, it is possible periodic retrievals from EM also contribute to retention. In addition to being an important strategy in normal function, this has the potential to complicate interpretations of mechanisms underlying behavior in WM tasks. Here, we provide evidence that EM affects behavior in simple WM tasks, even when WM is not disrupted.

To establish a marker for EM retrieval, 94 subjects studied multiple word lists, with each list associated with its own unique "context" picture. These words were later used as targets in a simple WM task: subjects first saw a target set composed of 4 words drawn from one of the lists; after an 18 second retention interval, a probe word appeared and subjects were asked whether the probe was not a member of the target set.

Given the short interval and the absence of overt distractions, it would ordinarily be assumed subjects perform the task by actively maintaining targets in WM, without reliance on EM. To test this, we investigated if presenting target words from a list triggers retrieval of the context (from EM) in which the list was studied, thus impacting performance. If context is not retrieved from EM, there should be no difference in responses to lures from the same context and distractors from other contexts. In contrast, if context is retrieved, it could assist in identifying targets and rejecting words from inconsistent contexts, and interfere with rejection of "lure" words from the same context as the targets.

We found same-context lures significantly slowed RT relative to different-context probes. This behavioral result suggests information retrieved from EM can affect participants' WM task performance.

To further test this hypothesis, we used MVPA on fMRI data to track reinstatement of the individual context images during retention intervals of the WM task. Reinstatement of target context correlated with RT to probes. On lure trials, this correlation was positive, indicating context information shared between targets and lures was distracting; on different context trials, this correlation was negative, indicating inconsistent context information between targets and different context words facilitated rejection. These fMRI results also support the hypothesis that EM retrievals occur during WM maintenance and impact performance in WM tasks.

Disclosures: **A. Novick:** None. **A.M. Bornstein:** None. **K.A. Norman:** None. **J.D. Cohen:** None.

Poster

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Topic: H.02. Human Cognition and Behavior

Title: Gluing memories via oscillations: Theta phase synchrony drives associative memory formation in humans

Authors: *A. CLOUTER, K. SHAPIRO, S. HANSLMAYR;
Univ. of Birmingham, Edgbaston, United Kingdom

Abstract: The objective of our experiments was to investigate the effects causal role of neural synchrony between visual and auditory processing regions on associative memory formation for multi sensory events.
Multisensory episodic memories rely on successfully binding elements that are processed in separate, specialised brain regions. The formation of episodic memories is thought to rely on the synchronization between distant brain regions in the theta frequency band. However, causal evidence for this idea from humans is missing. To provide such evidence we developed a novel multisensory memory paradigm where participants encode sound-movie associations. Modulating the luminance and amplitude of the videos and sounds independently allowed us to control the degree of phase synchrony between the auditory and visual cortex. We then further show in two experiments that memory for the sound-movie associations differs drastically depending on the degree of inter-sensory phase synchrony.
In the first experiment, in the encoding phase, all participants were shown short (3-second) videos that were luminance modified with a 4 Hz sine wave, with an accompanying audio clip that had been amplitude modulated with a 4 Hz sine wave. The phase offset (i.e., synchrony) between the audio clip and the video was 0, 90, 180, or 270 degrees. In a second experiment, the videos and sounds were modulated at 4 Hz, 1.7 Hz (delta), and 10.5 Hz (alpha). On each trial, participants rated how well the audio clip suited the contents of the video clip. Each of six blocks contained 16 audio-video pairings (four at each phase angle), and was followed by a brief distractor task and an associative recognition test.
Associations were better remembered in the synchronous compared to the asynchronous condition. This effect was specific to theta (i.e. 4Hz) and did not occur in a faster (10.5 Hz) or slower frequency (1.7 Hz). These findings suggest that episodic memory formation in humans relies on a theta specific synchronization mechanism.

Disclosures: **A. Clouter:** None. **K. Shapiro:** None. **S. Hanslmayr:** None.

Poster

744. Human Cognition and Memory V

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

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Topic: H.02. Human Cognition and Behavior

Support: This work was supported by JSPS KAKENHI grant numbers16H02839 and 15H01671

Title: Intersubject correlation analysis of students' brain activity during listening to teacher's explanation.

Authors: *Y. HIRAKO[1,2], T. KOIDE[2], T. ITO[3], S. SHIMADA[4];
[1]Meiji Univ., Tokyo, Japan; [2]Electrical Engin., Meiji graduate school, Science and technology, Japan; [3]Meiji university, School of Arts and Letters, Japan; [4]Electronics and Bioinformatics, Meiji university, Science and technology, Japan

Abstract: In this study, we investigated the mechanism of comprehending teacher's explanation by utilizing intersubject correlation (ISC) analysis of students' brain activity. ISC is calculated by utilizing general linear model (GLM) with one subject's brain activity as a model to see how similar the other subject's brain activity was.
Twenty-four right handed subjects participated in the experiment (one female, aged 21.3 ± 0.17 years, mean ± SD). Half of the subjects (N=12) listened to the explanation of a teacher about basic probability statistics in the recorded video. Another half listened to the equivalent explanation performed by another teacher in the recorded video. The hemodynamic responses in the bilateral cortical areas (9 x 9 square cm area each) were recorded by using 48-ch functional near-infrared spectroscopy (fNIRS) during explanation. The sampling frequency was 10 Hz. After the recording, the subject continuously (10 Hz) scored the rating of comprehensibility of the explanation by watching the movie again (0 - 100). The measured brain activity was analyzed by ISC analysis between two subjects who listened to the same teacher or different teachers. We also calculated Pearson's correlation between the ISC and the subjective rating of comprehensibility.
The ISC analysis showed significant ISCs in the right dorsolateral prefrontal cortex (DLPFC) (ch-38, BA9: $t(24) = 1.83$), the middle temporal gyrus (ch-46, BA21: $t(24)=3.70$), and the frontal eye field (ch-31, BA8: $t(24) = 1.71$). Among those, the right DLPFC showed a significant difference in ISCs between the subjects who listened to the same teacher and the subjects who listened to the different teachers (ch-38: $t(24) = 1.78$, $p<0.05$). The correlation analysis between the ISC and the subjective rating showed significant correlations at the left premotor cortex (ch-6, BA6: $r=0.24$, $p<0.05$) and the left angular gyrus (ch-21, BA39: $r=0.17$, $p<0.05$).
These results suggest that DLPFC is involved in comprehending teacher's explanation, by playing a role as working memory to translate verbal information into abstract numerical

representation, which likely covariates among students who listened to the same teacher's explanation.

Disclosures: **Y. Hirako:** None. **T. Koide:** None. **T. Ito:** None. **S. Shimada:** None.

Poster

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Support: NWO VENI 451.13.023

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NWO Brain & Cognition 433.09.208

ERC 339490

FP7 PITN-GA-2011-290011

Title: Reward and salience determine the precision of working memory encoding with different time-courses

Authors: ***P. C. KLINK**[1,2], D. JEURISSEN[1,3], J. THEEUWES[4], D. DENYS[2,1], P. ROELFSEMA[1,2,4];
[1]Netherlands Inst. For Neurosci., Amsterdam, Netherlands; [2]Academic Med. Center, Univ. of Amsterdam, Amsterdam, Netherlands; [3]Columbia Univ., New York, NY; [4]VU Univ., Amsterdam, Netherlands

Abstract: The general abundance of sensory information requires the brain to prioritize and select subsets of information for further processing and storage in working memory. Stimulus salience and reward expectations influence this prioritization but the underlying mechanisms are not well understood. We hypothesized that selective attention might act as a gating mechanism at the interface between sensory codes and memory storage. When multiple stimuli compete for representation in working memory, selective attention could bias this competition and affect the quality of memory encoding. Such a gating mechanism would furthermore have little effect on working memory during memory maintenance or retrieval. We tested the selective attentional gating hypothesis by investigating how stimulus saliency (contrast) and reward expectancy determine the precision of visual working-memory representations. Participants remembered the

orientation of three Gabor patches shown for 3s, and reproduced the orientation of one of them after a 2s memory interval. Reward expectancy was manipulated with color cues that indicated the amount of potential reward but had no predictive value about which stimulus would be probed. Bottom-up salience was modulated by changing stimulus contrast. Reward cues only affected memory precision when they were presented during the encoding phase of the task, not during maintenance or retrieval. Previously learned non-predictive color-reward associations had a similar effect on memory precision, but it gradually weakened over time. These reward-based priority effects required relatively long stimulus presentations as they disappeared when we shortened the encoding phase from 3s to 300ms. Interestingly, the influence of stimulus contrast on memory precision was oppositely related to encoding duration, with memory precision being affected with short but not with longer stimulus presentations. Our results provide new insight into how memory resources are flexibly distributed over potential memory targets, with selective attention acting as a dynamic gating mechanism at the interface between sensory and memory systems.

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Poster

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Topic: H.02. Human Cognition and Behavior

Support: National Science Foundation Graduate Research Fellowship

Title: Reward prediction errors enhance episodic memory

Authors: *N. ROUHANI[1], K. A. NORMAN[2], Y. NIV[2];
[1]Psychology, Princeton Univ., Princeton, NJ; [2]Psychology and the Princeton Neurosci. Inst., Princeton, Princeton, NJ

Abstract: Phasic dopaminergic signals are thought to track the degree to which actual outcomes are different from what was expected (a “reward prediction error” signal). These signals are critical for trial-and-error learning in the striatum. However, similar dopaminergic signals project to the hippocampus (Shohamy & Adcock, 2010), suggesting that prediction errors might influence the formation of episodic memories as well. Recent theoretical work has suggested that large prediction errors may serve to fractionate the stream of experience into separate memory traces (Gershman, Radulescu, Norman & Niv, 2014). Here we test the ensuing prediction that

more distinct traces will be formed in environments with higher risk (and therefore, larger prediction errors), leading to better memory fidelity. N=200 participants learned from trial and error which of two types of pictures, indoor or outdoor scenes, leads to higher rewards. Pictures were presented within two different contexts ('rooms'), with each room associated with a different degree of reward variance (risk) but matched in mean value, such that the rewards associated with pictures in one room gave rise to higher prediction errors than in the other room. On each trial, participants were shown an image, entered an estimate of how much that type of scene (indoor or outdoor) is worth on average, and were then re-presented with the image along with its reward. Participants learned the values for the scenes better in the low-risk than in the high-risk room, although they optimally reduced their learning rate in the high-risk environment. Conversely, a recognition test revealed that memory for high-risk scenes was better than that for low-risk, and that this effect was largely driven by better memory for images for which there was a greater absolute (or unsigned) prediction error at reward outcome. This finding suggests that enhanced memory for extreme outcomes (Ludvig et al., *J Beh Dec Making*, 2014) may actually be modulated by an absolute prediction error signal during learning. Current work is assessing whether reactivation of memories encoded at reward outcome (and within different 'risk' contexts) differentially biases decisions.

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Poster

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Topic: H.02. Human Cognition and Behavior

Support: NIH Grant AG017586

Wyncote Foundation

Title: A common, fine-grained code for object meaning in perirhinal cortex

Authors: ***A. R. PRICE**[1], M. BONNER[1], J. PEELLE[2], M. GROSSMAN[1];
[1]Univ. of Pennsylvania, Philadelphia, PA; [2]Washington Univ. in St. Louis, St. Louis, MO

Abstract: Many studies have examined object knowledge by studying the neural representation of object categories (e.g., tools versus animals), which often broadly differ on coarse features, such as shape, size, and texture. However, little is known about the neural mechanisms for encoding the fine-grained semantic attributes of specific objects within a semantic category. For

example, how do we know that a red apple is conceptually more similar to a green apple than to a blue apple? Here, we address this question by using a novel stimulus set that allowed us to leverage the natural statistics of object color information to investigate a neural code for object meaning. In an fMRI experiment, 16 subjects viewed images of objects that were systematically manipulated in color while performing an unrelated object detection task. The stimuli included three sets of specific objects (apples, leaves, and roses). The objects were each presented in five different colors (red, pink, blue, green, and yellow). For each object set, we created a semantic-similarity model based on the co-occurrence frequencies of color-object combinations from a large lexical corpus. This model predicts that "red apple" and "green apple" would have more similar neural representations than "red apple" and "pink apple" in brain regions that code high-level semantic information about objects. The semantic-similarity models were unique for each object category, and were orthogonal to perceptual models for shape or color similarity alone. Using representational similarity analysis of the multi-voxel patterns, we found that perirhinal cortex was the only region that significantly correlated with the semantic-similarity model ($p<0.01$). Next, we proposed that a key function of the semantic codes in this region is to provide a common understanding of object meaning across individuals. This predicts a specific functional architecture: neural codes in this region should be structured to provide a common ground between observers of the visual world. For example, a typical object like "red apple" should have a more similar neural instantiation across individuals than a less typical object like "blue apple." To test this hypothesis, we hyper-aligned each subject's data to a common, high-dimensional space (Haxby et al., 2011). Indeed, we found that perirhinal cortex was unique in containing population codes for which inter-subject similarity correlated with object typicality ($p<0.01$). Our results suggest that perirhinal cortex encodes high-level object knowledge and may instantiate a neural "common ground" for object meaning across individuals.

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Poster

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Topic: H.02. Human Cognition and Behavior

Support: NRF #2015-R1A2A2A04006136

Title: Goal-directed episodic memory decisions are reflected in the functional connectivity patterns

Authors: *Y. NAH[1], S. A. HUETTEL[2], I. G. DOBBINS[3], S. HAN[1];
[1]Dept. of Psychology, Yonsei Univ., Seoul, Korea, Republic of; [2]Dept. of Psychology and Neurosci., Duke Univ., Durham, NC; [3]Dept. of Psychology, Washington Univ. in St. Louis, St. Louis, MO

Abstract: Numerous functional magnetic resonance imaging (fMRI) episodic memory studies demonstrate that contrasting correctly recognized studied items (HIT) against correctly rejected novel items (CR), known as "Retrieval success effect", yields activation in distributed brain regions, including prefrontal, parietal, striatal and other regions. However, whether the interactivity of these distributed networks solely reflects retrieval judgment or goal-related decision has not been investigated thoroughly. We conducted fMRI studies using episodic Old/New recognition tasks to investigate the functional connectivity patterns within the 'retrieval success' networks during different recognition processes (HIT vs. CR) and manipulated the motivational significance of episodic memory decisions (i.e., performance-linked monetary reward and feedback). Nineteen participants judged whether each serially presented word is old (studied in encoding sessions) or new (never seen before) during retrieval. For the initial two runs, there was neither monetary reward nor performance feedback. For the last two runs, on the other hand, either correct "Old" or "New" responses were potentially rewarded in HIT-Incentive or CR-Incentive runs, respectively. Non-directional *F* test across the factors (Incentive condition × Feedback presence) revealed significant activations in several brain regions, including prefrontal, caudate, parietal and other regions, from which the task-dependent time-series were extracted. Pairwise cross-correlation coefficients of the extracted time-series across the networks were analyzed using functional connectivity multivariate pattern analyses (fcMVPA) with a linear support vector machine algorithm. The classification between HIT vs. CR iterated as a function of number of features included, and the peak accuracy (95%) was achieved. In addition, to explore how the connectivity patterns within retrieval success networks differ as a function of goal-dependent response type, fcMVPA were again applied in run 3 and 4, which showed peak accuracies of 95 to 100%. More importantly, connectivity patterns obtained during Incentive conditions in both runs (HIT-Incentive in run 3, CR-Incentive in run 4) showed similarly greater connectivity across the networks compared to standard responses in No-Incentive runs. These findings demonstrate that patterns of functional connectivity across nodes, previously known as retrieval success networks, reflect goal-congruent interactivity independent of the status of memory probes and suggest that participants preferentially construe HIT as intrinsically potential reward.

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Poster

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Canada Research Chairs Program

Title: Beyond slots and resources: an integrative approach to visual working memory

Authors: *R. SENGUPTA, J. K. TSOTSOS, S.-A. YOO, C. WLOKA, T. KUNIC;
Dept. of Electrical Engin. and Computer Sci., York Univ., Toronto, ON, Canada

Abstract: In recent years, research in the area of visual working memory has followed a few fixed avenues. One of the most influential formulations focuses on the capacity of visual working memory (VWM), an area which has been dominated by slot based frameworks (e.g., fixed slots, dynamic resource allocation with variable precision, etc.) (Luck et al., 1997; Ma et al., 2014). Interestingly, the debate has largely been within this paradigm. Research on capacity has largely progressed without a firm model of the actual content of working memory, relying rather on vague ideas of representation. Although it is widely held that attention acts as a gate to working memory, the actual mechanism of such a function largely remains elusive. In the current work we have developed an integrative account of visual working memory and attention. We have placed our model within the larger context of the selective tuning model of visual processing (Tsotsos et al., 2014) due to its comprehensive, biologically plausible, and computationally extensible framework. The content of VWM is the attentional sample collected by the visual processing hierarchy, and the capacity is determined by the resolution of the attentional sample and the computational constraints which apply to its subsequent maintenance in VWM (for instance during serial recall). Our model makes a number of predictions, including that the variability of working memory capacity in change detection experiments is determined by the physical size of the visual display. We have confirmed this prediction with a psychophysical experiment in which subjects performed a change detection task on a display which varied between 10x10 degrees to 12x12 degrees, and set sizes of objects varied between 2, 4 and 6. The idea of a fixed number of slots, and thus capacity, was originally supported by the poor performance at set sizes higher than 4 in working memory tasks (Cowan et. al., 2001). Our results show that for the larger display, change detection accuracy for 6 items was 15% greater than what was reported in Luck et. al. (1997), which is clearly outside what was traditionally considered the working memory capacity limit (Cowan et. al., 2001). Thus, we demonstrate that there is a display size dependency of performance which a classic slot view of working memory capacity cannot account for. Overall, we posit that the current framework provides a way to

break out of the slot based idea for working memory and move towards a more integrative approach to visual processing in general.

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Poster

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Topic: H.02. Human Cognition and Behavior

Support: Swedish Research Council

Title: Comparing fear conditioning using immersive virtual reality versus computer display

Authors: ***J. ROSÉN**, G. KASTRATI, F. AHS;
Dept. of Psychology, Uppsala, Sweden

Abstract: Fear conditioning in lab environments has proven to be an effective method when studying autonomic responses to threat. However the role of immersion has not been extensively researched in fear conditioning and extinction. Here we tested whether fear conditioning of virtual characters in an immersive virtual reality environment using head mounted display (HMD) was enhanced relative to fear conditioning in a lab environment using a traditional computer monitor. In both settings, one virtual character was displayed at a proximal and another character at a distant egocentric location, while being followed by the delivery of a mild electric shock (CS+). Two distance matched control stimuli were never paired with shock (CS-). Skin conductance responses (SCRs) were used as conditioning index. Results showed that proximal characters elicited greater SCRs than distant characters both when using immersive virtual reality and when using computer display. However, proximity enhancement of SCRs was greater in the virtual reality setting than in the lab setting. During fear conditioning CS differentiation was reduced at proximal location due to enhanced responding to the CS-, indicating slowed safety learning at proximal location. This effect was observed using either of the two display settings. Fear was extinguished to a similar degree when using immersive virtual reality and computer display. Results suggest that cued fear learning is similar when cues are displayed in immersed virtual reality as when cues are displayed at a computer monitor, but that distance manipulations have a greater effect on autonomic responses in immersive virtual reality.

Disclosures: **J. Rosén:** None. **G. Kastrati:** None. **F. Ahs:** None.

Poster

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Topic: H.02. Human Cognition and Behavior

Support: NIH R01 EY021755

NRSA F32 EY021999

Title: Disentangling remembered and perceived information in the full correlation matrix of human brain activity

Authors: *J. HUTCHINSON, Y. WANG, N. TURK-BROWNE;
Princeton Univ., Princeton, NJ

Abstract: Bringing to mind details from the past is often associated with the re-engagement of the same cortical regions that were involved when those details were initially perceived. Given this common substrate, how are we nonetheless able to distinguish internally and externally generated information? Here we explore the hypothesis that even if retrieval and perception share content, the neural sources of these representations differ, and thus measuring network engagement for these two processes might allow us to properly attribute our experience. To identify these divergent networks, we used full correlation matrix analysis (FCMA) — an unbiased, optimized technique for computing and decoding all pairwise correlations in the brain. In our study, 24 participants were trained to associate unique pairs of images (one face with one scene per pair) and were then presented with blocks of either face or scene images during fMRI. In the Perceive condition, they performed a male/female judgment on face images or a building/nature judgment on scene images. In the Retrieve condition, they performed the same set of judgments on the same sequences of images, but rather than judging the presented images, they judged the associated images from the other category based on memory. This design allowed us to hold visual stimulation constant while varying demands on internal vs. external processing. Applying a machine learning classifier to the full correlation matrix from each block, we were able to robustly decode with greater than 80% accuracy whether the block was from the Perceive or Retrieve condition. FCMA could also be used to identify voxels and regions whose connectivity with other regions was particularly informative. Moreover, the decoding and localization results could be compared with more traditional, activity-based multivariate pattern analysis (MVPA) approaches, and with behavior in the different tasks. The results provide insight into how the same representational space can be used by both internally and externally driven processes without necessarily leading to failures of reality monitoring. More generally, they highlight the value of quantifying cognitive processes in terms of connectivity patterns, in

addition to activity patterns and overall activity, when seeking to understand brain mechanisms and dynamics.

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Poster

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Support: NIH Grant F32 HD 078223

Title: Online measures of perceptual integration predict statistical learning

Authors: *L. BATTERINK, K. A. PALLER;
Northwestern Univ., Evanston, IL

Abstract: Statistical learning allows learners to discover structure in the environment, as occurs during speech segmentation. Statistical learning may be divided into at least two dissociable components, perceptual binding of individual stimulus units (e.g., syllables) into integrated items (e.g., words), and encoding of these extracted items into long-term memory. Previous studies have generally focused on the outcome of statistical learning by assessing performance on post-learning tasks, and thus have not dissociated these two components. The goal of the present study was to characterize the perceptual component of statistical learning, examining whether differences in learners' online perception of input predicts performance on post-exposure learning tasks. Participants were exposed to streams of repeating trisyllabic nonsense words ("structured" condition) and of randomly concatenated syllables ("random" condition). Online learning was indexed by an EEG-based measure that quantified neural entrainment at the frequency of the repeating words, reflecting whether learners subjectively experienced individual elements or integrated items as the basic perceptual units of the speech stream. After exposure, statistical learning was assessed offline using both an explicit rating task and a reaction-time task. In the structured condition, neural entrainment to the trisyllabic words (1) was higher than in the random condition, (2) increased as a function of exposure and, (3) predicted performance on the post-exposure reaction time task. Unexpectedly, neural entrainment to trisyllables in the random condition also predicted post-exposure task performance. These results suggest that neural measures are sensitive to an individual's ability to seek out underlying patterns in the environment, which is a key predictor of statistical learning success. Our findings also provide evidence that the perceptual binding of individual stimuli into integrated items is a critical

component of statistical learning and an important source of variability in statistical learning performance.

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Poster

744. Human Cognition and Memory V

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Program#/Poster#: 744.27/KKK15

Topic: H.02. Human Cognition and Behavior

Support: KFAS Fellowship to H.L.

Title: Parietal memory reactivation and retrieval-induced modification of long-term memories

Authors: ***H. LEE**[1], R. SAMIDE[2], F. R. RICHTER[3], B. A. KUHL[2];
[1]Dept. of Psychology, New York Univ., New York, NY; [2]Dept. of Psychology, Univ. of Oregon, Eugene, OR; [3]Dept. of Psychology, Univ. of Cambridge, Cambridge, United Kingdom

Abstract: Lateral parietal cortex, specifically angular gyrus (ANG), has consistently been implicated in long-term memory retrieval: activity in ANG increases with vivid remembering, and the contents of reactivated memories can be decoded from ANG activity patterns. However, understanding the functional significance and nature of memory reactivation within ANG remains an important objective. For example, does reactivation within ANG functionally differ from reactivation in occipito-temporal cortex (OTC)? Here, we addressed this issue by asking whether reactivation in ANG and OTC predicts retrieval-induced strengthening or modification of memories. We conducted a three-phase fMRI experiment. In phase 1 (learning phase), subjects studied associations between words and pictures of faces, scenes, or objects. During phase 2 (retrieval practice), half of the previously learned words were presented alone and subjects retrieved the picture associated with each word. Words were presented three times each and subjects rated the vividness of the retrieved memory on each trial. In the final phase (test phase), which took place outside of the scanner, subjects were tested on their recognition memory for pictures. There were three types of test pictures: novel, studied, and critical 'lure' pictures that were perceptually similar and conceptually identical to the studied pictures. For each picture, subjects indicated whether it was 'old' or 'new' and how confident they were. Behavioral results indicated that retrieval practice increased recognition accuracy for the pictures, but that higher accuracy occurred via an increase in hit rate and false alarms (with the increase in hit rate being larger). This effect was particularly evident when subjects reported high vividness during retrieval practice. Consistent with prior findings, vivid remembering (during

retrieval practice) was associated with higher univariate activation and pattern-based reactivation in ANG and OTC. However, reactivation in ANG vs. OTC differentially predicted performance on the post-test: reactivation in OTC—but not ANG—was associated with a reduced rate of false alarms to critical lures. Thus, while activation and pattern-based information in ANG strongly scaled with vivid remembering, ANG reactivation did not protect against the increased false alarm rate that was associated with retrieval practice. These findings suggest that ANG representations are more likely to be gist-based or semantic in nature than representations in OTC and that by separately considering reactivation in each region, predictions can be made about how the act of retrieval influences a memory.

Disclosures: **H. Lee:** None. **R. Samide:** None. **F.R. Richter:** None. **B.A. Kuhl:** None.

Poster

745. Human Cognition: Individual Differences I

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Topic: H.02. Human Cognition and Behavior

Support: University of Maryland SPARC

Title: Computational model and erp enabled prediction of single trial behavior on a numerical comparison task

Authors: *R. W. PRATHER, S. HEVERLY-FITT;
Human Develop. & Quantitative Methology, Univ. of Maryland, College Park, MD

Abstract: We demonstrate how a computational model can predict single-trial behavior for individual participants completing a numerical comparison task. Participants performed a non-symbolic numerical comparison task while reaction time, response, and electrophysiological (EEG) data were recorded. Mean ERP amplitude within the time window of 280-380ms was extracted for each trial. Consistent with existing literature on numerical processing we find a distance effect with both behavioral and neural measures. Participant accuracy correlated positively with the proportional distance between the two values being compared. We also found a significant correlation between parietal ERP amplitude (P3 and P7 channels) and the distance between the comparisons being made. We then used a multilayered dynamical systems model with evolutionary algorithm updating to predict behavior for each participants performance on a trial-by-trial basis. Each participant's data was modeled with an independent instantiation of the model. The initial model training included the first two thirds of each participant's data. Model input included trial response, reaction time and average ERP amplitudes for parietal channels.

The training comprised of multiple generations of an evolutionary algorithm in which model specifications were adjusted to best fit the individual participant's data. Accuracy in initial model fit to individual participant responses ranged from 82% to 95%. Accuracy for reaction time fit ranged from a mean deviation of 150ms to 400ms. The best fit model specifications for each participant were then used to predict novel data, the remaining 1/3 of trials. The computational models were able to generalize to novel data as well as data used for the initial optimization. We discuss leveraging individualized prediction of on number comparison performance to symbolic arithmetic tasks, an important educational and developmental outcome.

Disclosures: R.W. Prather: None. **S. Heverly-Fitt:** None.

Poster

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NIH NS088590

Mallinckrodt Institute of Radiology

Child Neurology Foundation

American Psychological Association

Washington University Dept. of Psychological and Brain Sciences

Title: Characterizing parietal memory network responses under incidental memory task conditions in highly-sampled individual subjects using fMRI

Authors: *A. W. GILMORE[1], S. M. NELSON[7,8,9], T. O. LAUMANN[2], J. J. BERG[1], D. J. GREENE[3,4], E. M. GORDON[7,8], A. L. NGUYEN[2], M. ORTEGA[2], R. S. COALSON[3,2], B. L. SCHLAGGAR[3,2,5,4,6], S. E. PETERSEN[1,3,2,5], N. U. F. DOSENBACH[2], K. B. MCDERMOTT[1,3]; [1]Psychological and Brain Sci., Washington Univ., Saint Louis, MO; [2]Neurol., [3]Radiology, [4]Psychiatry, [5]Anat. and Neurobio., [6]Pediatrics, Washington Univ., St. Louis, MO; [7]VISN 17 Ctr. of Excellence for Res. on Returning War Veterans, Waco, TX; [8]Ctr. for Vital Longevity, Univ. of Texas at Dallas, Dallas, TX; [9]Psychology and Neurosci., Baylor Univ., Waco, TX

Abstract: Recently, a sparse network of regions in parietal cortex has been associated with encoding and retrieval processes in human long-term memory. Activity within this "parietal memory network" (PMN) appears to follow three main response patterns depending on one's history with a given stimulus (Gilmore et al., 2015): 1) PMN regions deactivate relative to a resting baseline for newly-encountered stimuli (as during a typical study phase); 2) PMN regions show repetition enhancement effects (i.e., increase in activity) across item repetitions; 3) PMN regions activate above resting baseline levels for stimuli that have been observed multiple times (as during a typical retrieval phase). BOLD responses in PMN regions are thought to reflect the degree to which a stimulus is perceived to be novel or familiar, though the relative contributions of familiarity per se and of attention to that familiarity are not yet well understood. In part, this is because our understanding of the PMN is based on explicit memory experiments, in which subjects are required to either intentionally encode information in preparation for a later test, or are explicitly directed to orient to their history with a given item. The degree to which the PMN responds similarly under incidental memory conditions—in which no direct test of memory is conducted—is unknown, and may be helpful in understanding how different processes affect PMN activity. This was explored in the present work.

Ten highly-sampled subjects underwent fMRI scanning across ten different days. Each day, subjects performed an incidental memory task, in which they were instructed to make semantic decisions about stimuli that appeared multiple times in the course of a single session (e.g., whether a word referred to an abstract or concrete concept). Sufficient data were collected to conduct analyses on the level of individual subjects. Whole brain voxelwise analyses of BOLD activity associated with different stimulus presentations revealed regions exhibiting both repetition enhancement and repetition priming. Targeted interrogation of PMN regions revealed that they deactivated as predicted during initial stimulus presentations, and also showed repetition enhancement across subsequent presentations. However, PMN regions did not activate above baseline for familiar stimuli, and instead remained near baseline activity levels.

The current data suggest that processes related to stimulus salience or attentional capture may account for repetition enhancement above baseline in PMN regions, whereas processes driven by novelty or familiarity can produce deactivation and lesser degrees of repetition enhancement.

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Poster

745. Human Cognition: Individual Differences I

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Support: ImPACT Program of Council for Science, Technology and Innovation (Cabinet Office, Government of Japan)

JSPS KAKENHI Grant Number JP26730146

Title: Individual representation of large scale brain network related to creative insight: Voxel-Based Morphometry <and> resting-state functional connectivity analyses

Authors: *T. OGAWA[1], T. AIHARA[2], T. SHIMOKAWA[2], O. YAMASHITA[2];
[1]ATR, [2]NIA, ATR, Kyoto, Japan

Abstract: Creative cognition is commonly agreed to play an essential role in the development of human civilization <and> cultural life. With use of recent neuroimaging techniques, neuroscientists of creative cognition have been studying on creative insight <and> domain-general/specific creative thoughts. However, an insight-specific network <and> its individual difference of broad-age ranged adults have been rarely investigated using both structural <and> functional neuroimaging techniques. Here, we focused on large-scale network associated with insight problem solving. We defined insight problem solving as including three characters: i) a solver needs to discover a single solution with convergent thinking, ii) a solver needs to change representation of a problem, iii) a solver suddenly moves from a state of not knowing a solution to a state of knowing a solution. We conducted an MRI experiment (T1-weighted image <and> 10-min resting-state fMRI scanning) <and> multimodal behavioral experiments to measure the cognitive function on 101 healthy subjects ranged in age from twenties to sixties. In the Voxel-Based Morphometry analysis of the T1-weighted images (76 participants), we identified seed brain regions where the gray-matter volume (GMV) were positively correlated with scores of insight. We applied the seed-based functional connectivity analysis of resting state fMRI to whole brain voxels. We found that the GMV in the bilateral precunei, the right middle cingulate cortex (*R*-MCC), the right superior parietal lobule (*R*-SPL) <and> the left middle temporal gyrus (*L*-MTG) were positively correlated with IPS score. According to the obtained seed regions, we extracted a task-positive network consisting of precunei, MCC, <and> superior frontal gyrus (SFG), <and> a task-negative network consisting of left MTG, superior temporal gyrus (STG), insula, right MTG, right fusiform, STG <and> bilateral precunei. This is the first study to identify the large-scale networks associated with creative insight. Our results support that individual creative insight ability was correlated with altered GMV involved in cognitive control regions, <and> that the insight score was associated with the strength of resting state functional

connectivity between the precunei <and> other brain regions related to default mode or language processing. These findings are comparable to the previously reported studies on the creative cognition such as domain-general creative thought.

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Poster

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McDonnell Foundation Collaborative Activity Award

Title: Effects of global signal regression (GSR) and motion scrubbing are essentially similar between and within subjects.

Authors: *B. ADEYEMO[1], B. SEITZMAN[1], S. E. PETERSEN[1,4,2,3];
[1]Dept. of Neurol., Washington Univ. Sch. of Med., Saint Louis, MO; [2]Dept. of Radiology, [3]Dept. of Neurosci., Washington Univ. Sch. of Med., St. Louis, MO; [4]Dept. of Psychological and Brain Sci., Washington Univ. in St. Louis, St. Louis, MO

Abstract: Head motion is a pernicious problem for analyses involving resting state functional correlation (RSFC) data. Motion-related effects include motion outlier spikes directly associated with small motions and global artifacts found in greater abundance in scans with frequent movements. Because these artifacts produce systematic and spurious positive correlations, differences can arise when groups systematically differ with regard to these artifacts. While many have accepted the spiking artifact and have taken steps to address this (e.g. removal of affected frames, “scrubbing”), many still have yet to concede the importance of the global artifacts, in part because of potentially important global signal differences at the individual or group level.
In an attempt to address effects at a subject level we created two subgroups (N=25 each) from

the HCP database of RSFC data. All subjects had two separate sessions of resting state functional imaging (RSFC). For the LL group, both scans were associated with low amounts of motion. For the LH group, one scan had relatively low motion, the other had significant motion. The main assessment was the number of statistical differences between cells in the adjacency matrices sets of scans. We then assessed the effects, both across groups and within each of the two groups, when applying different combinations of scrubbing and global signal noise reduction algorithms. When comparing the lowest of the LL sessions with the high motion session of the LH group, thousands of differences were found. Removal of high motion frames (scrubbing), decreased the difference by about 29%, GSR by about 71%, and the combination about 77%. Similar measurements within the LH group were 28%, 87% and 90% respectively. Comparing the matrix effects for the between and within conditions showed considerable similarity: matrices correlate at 0.6.
Thus, the great bulk of the differences found in uncleaned data are due to artifact, and group and individual differences must be viewed with extreme caution when either or both GSR and motion scrubbing are not performed.

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Poster

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Topic: H.02. Human Cognition and Behavior

Title: Binaural beats for enhancing cognitive functions in healthy individuals

Authors: *G. B. PATRUDU;
Andhra Med. Col. & King George Hosp., Visakhapatnam, India

Abstract: Currently there is a surge in interest on research into the effect of binaural beats on cognitive functions. This review summarizes the results of studies on the effect of binaural beats on cognitive functions. Binaural beats have been shown to improve phase synchronization which is vital for cognitive processes. Binaural beats in the alpha (10 Hz) and theta (4 Hz) frequency range enhanced alpha-band oscillation synchrony between the auditory cortices during auditory stimulation (Solca et al., 2016). Also an increased temporo-lateral phase synchronization was observed due to 5Hz binaural-beat stimulation (Becher et al., 2015). Like wise, they have been shown to enhance attention.On a global local task listening to Gamma frequency binaural beats produced more attentional focussing(Colzato et al., 2015).In another study, Gamma frequency binaural beats eliminated the Attentional Blink, but only in individuals with low spontaneous

eye-blink rates (Reedijk et al., 2015). Similarly, individuals with low Eye Blink Rates mostly benefitted on task requiring creativity (Alternate uses task) from alpha binaural beat stimulation, while individuals with high Eye Blink Rates were unaffected or even impaired by both alpha and gamma binaural beats (Reedijk et al., 2013). Ultrashort duration of presentation (2 min)of steady state binaural beats were not sufficient to alter vigilance or entrain cortical frequencies at the two bands examined Theta (7 Hz) and Beta (16 Hz) in a study by Goodin et al. (2012).In a study by Kennel et al., (2010), listening to the binaural beats participants reported subjectively experiencing less problems associated with inattention during the study period. Auditory stimulation over a long time at a frequency of 5 Hz(theta) increased the capacity of immediate verbal memory(Ortiz et al., 2008). However with 7hz theta frequency for only a single session of 30 minutes showed decrease in immediate verbal memory recall (Wahbeh et al., 2007). In a study by Lane et al. (1998) listening to Binaural beta beats while performing visual vigilance task improved performance .Overall, the results of the studies on the effect of binaural beats on cognitive functions look positive. However , conflicting findings in some studies seem to be due to individual variations or predispositions of the study participants and the duration of application of binaural beats . More studies have to be done taking these factors into consideration to study effectively the influence of binaural beats on various cognitive functions.

Disclosures: G.B. Patrudu: None.

Poster

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Program#/Poster#: 745.06/KKK21

Topic: H.02. Human Cognition and Behavior

Title: High-fidelity descriptions of the brain networks of individuals with and without Traumatic Brain Injury using fMRI

Authors: *E. M. GORDON[1,2], B. S. GARY[1], R. M. SWEIDAN[1], T. O. LAUMANN[3], D. J. GREENE[4,5], J. J. BERG[6], A. W. GILMORE[6], K. B. MCDERMOTT[4,6], S. E. PETERSEN[3,4,6,7], B. L. SCHLAGGAR[3,4,5,7,8], N. U. F. DOSENBACH[3], S. M. NELSON[1,2,9];
[1]Ctr. of Excellence for Res. on War Veterans, Waco, TX; [2]Ctr. for Vital Longevity, Univ. of Texas at Dallas, Dallas, TX; [3]Neurol., [4]Radiology, [5]Psychiatry, [6]Psychological and Brain Sci., [7]Neurosci., [8]Pediatrics, Washington Univ. Sch. of Med., St. Louis, MO; [9]Psychology and Neurosci., Baylor Univ., Waco, TX

Abstract: Lasting cognitive and behavioral effects of Traumatic Brain Injuries (TBI) are common among post-deployment military Veterans who have suffered head injuries. Such

symptoms are related to the presence of diffuse axonal injury resulting from shearing forces; this damage impairs normal communication between distant brain regions, effectively disrupting brain networks critical for cognitive function. However, studying how TBI disrupts brain networks has been challenging due to inter-individual variability in both the injuries sustained and the networks themselves. Recent work by Laumann et al. (2015) has demonstrated that the brain networks of individuals can be characterized with high fidelity if many hours of resting state functional connectivity MRI (rs-fcMRI) data are collected in each individual. This work found that the functional network organization of individual brains are significantly more complex than group-average networks. Here, we apply a similar high-data rs-fcMRI approach to study TBI-related effects on brain networks. We collected 2-3 hours of rs-fcMRI data from 5 US Military Veterans with a history of TBI (four with mild TBI only; one with moderate TBI) and from 2 Veterans with no TBI history. Functional connectivity analyses characterized both the spatial topographies of brain networks and the strength of functional connections within and between those networks. Similar to Laumann et al (2015), all subjects' individualized brain networks contained small network pieces that did not correspond to any network features present in group average data. Notably, network maps of TBI patients were not systematically different from those of either non-TBI Veterans or healthy non-Veteran individuals. However, the magnitudes of both positive and negative functional connections between and within networks were reduced in the TBI patients compared to the non-TBI participants. These results suggest that TBI-induced disruption of white matter may reduce absolute functional connectivity strength, but leave network shapes unchanged. More broadly, these results indicate the possibility that the individual-specific spatial topographies of functional brain networks may be relatively insensitive to damage that impairs connectivity within and between these networks.

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Poster

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Topic: H.02. Human Cognition and Behavior

Support: Ellison Foundation

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Title: Deep dynamic phenotyping of the individual: tracking within-subject variability in social and emotional behavioral profiles using smartphones

Authors: *M. SHERMOHAMMED[1], I. J. BARNETT[2], M. V. KIANG[2], R. L. BUCKNER[1,3,4], J. T. BAKER[3,4,5], J.-P. ONNELA[2], L. H. SOMERVILLE[1];
[1]Psychology, Harvard Univ., Cambridge, MA; [2]Harvard T.H. Chan Sch. of Publ. Hlth., Boston, MA; [3]Massachusetts Gen. Hosp., Boston, MA; [4]Harvard Med. Sch., Boston, MA; [5]McLean Hosp., Belmont, MA

Abstract: Extensive longitudinal phenotyping in the individual creates novel opportunities to measure physiology and brain states in relation to real world changes in social and emotional behavior. To explore this possibility, we tailored a custom smartphone research platform called Beiwe (Onnela & Rauch, 2016) to capture shifts in behavior and psychological state. Information about movement was passively captured using the phone's accelerometer, about location using GPS, bluetooth, and wifi, and about social interactions using text and call logs. In addition to these passive measures, participants completed 37 daily questions related to their physical, emotional, and social experiences that day. These combined measures allowed detailed monitoring of four individual participant's daily changes over a period of four months in mood, stress levels, energy, appetite, physical activity, and social engagement. Data-driven analysis techniques probed the relationships between measures within and across data types. Results revealed meaningful relationships between measures, such as the clustering of self-reported affect by valence and subject-specific relationships between affective states and social behaviors, suggesting that real daily changes in psychological state are captured. This information provides measurement of idiosyncratic psychological profiles. One of the primary purposes of this endeavor is to serve as a proof-of-concept of intensive daily behavioral probes. We found that subjects were able to maintain a high rate of compliance, supporting the feasibility of such a paradigm moving forward. Parallel work will integrate these behavioral and psychological measures with functional MRI data on the same individuals to examine day-to-day shifts in the organization of functional brain networks in healthy individuals, as well as individuals under stress, suffering from mood disorders, and at different points across the developmental course.

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Poster

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Canadian Institute of Health Research Banting Fellowship

Title: Deep dynamic phenotyping of the individual: Circuit dynamics underlying longitudinal fluctuations in mood and cognition in patients with bipolar disorder

Authors: *J. T. BAKER[1,2,3], R. M. HUTCHISON[4], R. M. BRAGA[4,1,3,5], J. A. NIELSEN[4,1,3], L. FARFEL[4], M. E. MAROTTA[4], N. MUELLER[2], R. J. JUELICH[2], J.-P. ONNELA[6], D. ONGUR[1,2], R. L. BUCKNER[4,1,3];
[1]Harvard Med. Sch., Boston, MA; [2]McLean Hosp., Belmont, MA; [3]Massachusetts Gen. Hosp., Boston, MA; [4]Harvard Univ., Cambridge, MA; [5]Imperial Col., London, United Kingdom; [6]Harvard T. H. Chan Sch. of Publ. Hlth., Boston, MA

Abstract: Longitudinal studies of neural circuit changes in individuals suffering from bipolar disorder are essential to advance our understanding of this inherently unstable condition, which has typically been studied in cross-sectional cohorts comparing groups with illness to controls. Until recently, longitudinal brain imaging coupled with dense behavioral characterization was not feasible due to the high burden of scanning and phenotyping instruments that depend on office visits. Smartphones are now ubiquitous devices for behavioral data acquisition in naturalistic settings, and together with the technological refinements in accelerated brain imaging, allow for these crucial studies to proceed. Here we describe a pilot study to collect behavioral, hormonal and anatomical / functional MRI changes at a time-scale during which significant fluctuations in mood and cognition are likely to occur in individuals with severe mental illness. Allostatic and homeostatic mechanisms at play within the medial frontal cortex, hippocampus, and related limbic structures could impinge upon the anatomy and functional

architecture of these systems, allowing us to characterize biological dynamics that underlie changes in mood and cognition. In four adults with a psychotic disorder (bipolar disorder with psychosis, schizoaffective disorder or schizophrenia), a brief core battery of functional tasks was acquired on separate days simultaneously with physiological measures (heart rate, respiration, GSR) and eyetracking (pupil size, position, eye closures). Visuomotor (120s), face matching (120s), rule switching (108s), 2-back (108s), mental rotation (108s) microtasks and a passive fixation "rest" (844s spread over two runs) task were collected. Simultaneous multi-slice sequences were used (Siemens 3T Prisma, SMS=5, TE=32.6ms, TR=1.0s, vox=2.4mm). Scanning was spaced over one year (up to 15 sessions per participants), with adaptive MRI sampling to more densely capture any changes in brain anatomy or function around periods of illness fluctuation. We found that patients remained compliant with daily surveys and monthly scan protocols even during periods of low mood and motivation. Relationships between smartphone-derived metrics of mood, energy, and cognition were examined in relation to menstrual cycle, periods of exogenous stress, and changes in treatments. These studies demonstrate proof-of-concept for applying a deep dynamic phenotyping approach in individuals with severe mental illness, suggesting a powerful novel paradigm for evaluating the effects of interventions on brain structure and function, and ultimately behavior.

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Poster

745. Human Cognition: Individual Differences I

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Topic: H.02. Human Cognition and Behavior

Title: Allelic variations in PLP1 and CNTN1 modulate interhemispheric integration

Authors: *S. OCKLENBURG[1], W. M. GERDING[1], L. ARNING[1], E. GENÇ[1], J. T. EPPLEN[1], O. GÜNTÜRKÜN[1], C. BESTE[2];
[1]Ruhr-University of Bochum, Bochum, Germany; [2]Cognitive Neurophysiol., TU Dresden, Dresden, Germany

Abstract: Objective of the study:
The corpus callosum is the largest white matter tract in the human brain. Its main function is the integration of sensory, motor and cognitive information between the two hemispheres. Interestingly, interhemispheric communication during demanding cognitive tasks shows

pronounced inter-individual variation. The molecular mechanisms underlying these differences between individuals are largely unknown. Since the speed of axonal conduction depends on both the diameter of the axon and its myelination, genes involved in myelination are likely to influence the speed of interhemispheric integration. The aim of the present study was to investigate the role of allelic variations in myelin genes on interhemispheric integration.
Materials and Methods:
Overall, 453 healthy adults were genotyped for 18 single nucleotide polymorphisms (SNPs) in six myelin-related candidate genes (PLP1, GPM6A, MOG, MBP, CNTN1 and MOBP). To understand the possible genetic impact of these myelin genes on interhemispheric integration, the Banich-Belger Task, a widely used behavioral paradigm to assess interhemispheric integration, was used.
Results:
We replicated the typical pattern of results in the Banich-Belger Task, supporting the idea that performance on cognitively demanding tasks is enhanced when cognitive processing is distributed across the two hemispheres. Moreover, allelic variations in the proteolipid protein 1 gene PLP1 and the contactin 1 gene CNTN1 correlated with the extent to which individual performance was enhanced by interhemispheric integration.
Conclusion:
The results show that allelic variations in PLP1 and CNTN1 modulate the efficacy of transcallosal transmission. Genetic variation in myelin genes possibly affects the microstructure of the corpus callosum by altering oligodendrocyte structure, in turn modulating interhemispheric integration.

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Poster

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Herchel Smith-Harvard Undergraduate Science Research Program

Title: Deep dynamic phenotyping of the individual: Dynamics associated with stress and transitions of college life

Authors: *G. COOMBS III[1], M. P. HIND[1], J. A. NIELSEN[1,2], J.-P. ONNELA[3], R. L. BUCKNER[1,2,4];
[1]Harvard Univ., Cambridge, MA; [2]Massachusetts Gen. Hosp., Charlestown, MA; [3]Harvard T.H. Chan Sch. of Publ. Hlth., Boston, MA; [4]Harvard Med. Sch., Boston, MA

Abstract: Many questions about behavior and brain function focus on changes over time including responses to mood states and stress. Commonly occurring transitions during adolescence, in particular when starting college, are periods of high stress and increased risk for developing mental illness. 63% of college students say daily stress significantly interferes with their work. Thus, it is important to understand how college students adapt to increased academic and social pressures, and how brain and behavioral states change over time within individuals in response to real-world stressors. Sixteen healthy college students were deeply phenotyped over the course of 7 months (1 academic semester and breaks). Participants completed a daily 40 item survey about their physical, emotional, and social experiences, including individually tailored questions about specific stress-relief behaviors (e.g., eating, exercise, etc.). Objective measures of sleep, physical activity, and sociability were measured using an actigraphy monitor and a custom smartphone research platform (Beiwe; Onnela & Rauch, 2016). Finally, participants completed a brief battery of tasks selected to probe different cognitive domains, including face perception, reward, and executive function. To understand how stress-related states affect behavior and cognitive performance, participants completed the battery before and after real-world stressors, specifically the days immediately surrounding difficult exams for a total of 12 sessions throughout the semester. Results revealed meaningful relationships between measures, such as increases in self-reported stress and time spent cramming leading up to exams. Additionally, in some subjects, increases in stress and cramming were related to both subjective and objective measures of sleep quality, as well as other behaviors. This information provides measurement of idiosyncratic psychological profiles. Parallel work will integrate these behavioral and psychological measures with functional MRI data on individuals, including those enrolled in difficult courses and incoming freshmen as they transition to college, to examine stress-related shifts in the organization of functional brain networks and their relationship to real-world behaviors. Dense longitudinal study of the individual provides a foundation to better understand brain states and network interactions that change over time in response to life transitions and environmental stressors.

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Poster

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Canadian Institute of Health Research Banting Fellowship

Title: Deep dynamic phenotyping of the individual: a platform for precision neuroscience

Authors: *R. L. BUCKNER[1,2,3], R. M. BRAGA[1,2,4], R. M. HUTCHISON[1], M. SHERMOHAMMED[1], G. COOMBS[1], I. J. BARNETT[5], M. V. KIANG[5], L. FARFEL[1], M. E. MAROTTA[1], R. W. MAIR[1,2,3], T. M. O'KEEFE[1], J. MEICKLE[1], H. LIU[2,3], L. H. SOMERVILLE[1], J.-P. ONNELA[5], J. T. BAKER[2,3,6];
[1]Harvard Univ., Cambridge, MA; [2]Massachusetts Gen. Hosp., Boston, MA; [3]Harvard Med. Sch., Boston, MA; [4]Imperial Col. London, London, United Kingdom; [5]Harvard T.H. Chan Sch. of Publ. Hlth., Boston, MA; [6]McLean Hosp., Belmont, MA

Abstract: The past era of human neuroscience has focused on group-averaged data acquired from temporarily isolated or sparse samples of the brain and behavior. Mobile aspects of the human condition have been underexplored including dynamic responses to transient life stressors, illness, administered pharmacology, childbirth and unexplained variation that is characteristic of adolescence and mental illness. Research focused on the group also emphasizes methods that may miss idiosyncratic features of the individual's brain and unique path through life. In addition to leaving major areas of human neuroscience uncharted, these emphases contribute to the challenge of translating functional neuroimaging approaches into clinical tools. Recent reports from Chen R et al (2012 Cell) and Poldrack RA et al. (2015 Nat Commun) provide proof-of-concept demonstrations that human biology, neuroimaging, and behavior can be intensely tracked over extended periods in the individual. Here we expand on these pioneering efforts by constructing and demonstrating a platform to extensively study individuals over time

using a combination of low-burden neuroimaging techniques that employ 'micro-tasks' and optimized brief acquisition sequences, real-world behavior and sleep tracking using active and passive features of smartphones and wearables, measures of hormones through saliva, and daily recorded voice diaries. Data and quality control procedures from three initial studies illustrate the potential of deep dynamic phenotyping as well as reveal new challenges. New challenges include constructing numerous versions of tasks that minimize habituation effects, keeping participants engaged, monitoring safety (including monitoring effects on hearing), and maintaining data security. We were particularly surprised by the variability in arousal across MRI sessions within a participant that may have contributed to miss-estimation of trait-attributed variance in prior studies. In addition to initial results, we also illustrate the neuroinformatics tools that have been constructed to handle these data which extend from the Brain Genomics Superstruct (GSP) project (Holmes et al., 2015 Sci Data) with refactoring to accommodate thousands of data entries from a small number of participants in contrast to a small number of data entries from thousands of participants.

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Poster

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Title: Left-ward structural asymmetry in visual word form area favors verbal Stroop performance

Authors: ***A. VALLESI**[1], I. MAZZONETTO[2], A. BERTOLDO[2];
[1]Neurosci., [2]Dept. of Information Engin., Univ. of Padova, Padova, Italy

Abstract: The Visual Word Form Area (VWFA) is thought to integrate feed forward visual processing with top-down influences from more anterior areas, such as those responsible for phonological and semantic processing during written word recognition. Intriguingly, activity in

the left VWFA is influenced by Stroop interference. In the light of these previous findings, we tested whether structural MRI asymmetries in the whole-brain, and in particular in the VWFA, would predict inter-individual differences in combatting Stroop interference. To check for the specificity of the results, we employed both a verbal Stroop task and a spatial one. Three groups (111 healthy young adults; F=80; mean age: 23 years, all right-handed) were scanned with 3T MRI at different sites. Surface area estimation and statistical analysis were carried out on T1-weighted images using an automated surface-based method in FreeSurfer. Participants' hemispheres were registered to a symmetric template (smoothing: 15 mm FWHM) and a Laterality Index (LI) for the area of each vertex was computed for each participant. The correlation of the cortical area LI with a Stroop interference measure (RT incongruent-congruent) was assessed at each vertex by means of a general linear model with a whole-brain non-parametric cluster-based multiple comparisons correction, after adding 3 covariates coding for the different sites. The results showed that an asymmetrically more pronounced surface area on the left hemisphere in a cluster including the VWFA was significantly correlated with lower Stroop interference in the verbal task only. These results confirm and extend previous functional MRI findings, by showing a role of the VWFA for higher-level processes based on word reading, including the suppression of word reading when this is required by the task. In particular the findings show that individuals with more left-lateralized VWFA have an advantage in combatting verbal Stroop interference suggesting, on the flip side, that a bigger VWFA on the right hemisphere may be detrimental for this process.

Disclosures: **A. Vallesi:** None. **I. Mazzonetto:** None. **A. Bertoldo:** None.

Poster

745. Human Cognition: Individual Differences I

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Mallinckrodt Institute of Radiology

McDonnell Center for Systems Neuroscience

Hope Center for Neurological Disorders

Title: Common and unique topological variants: Individual differences in the functional organization of the resting human brain

Authors: *B. A. SEITZMAN[1], T. O. LAUMANN[1], B. ADEYEMO[1], E. M. GORDON[6,7], A. W. GILMORE[8], J. J. BERG[8], M. ORTEGA[1], A. NGUYEN[1], D. J. GREENE[2,3], K. B. MCDERMOTT[8,3], S. M. NELSON[6,7,9], B. L. SCHLAGGAR[1,3,4,2,5], N. U. F. DOSENBACH[1], S. E. PETERSEN[1,8,3,5];
[1]Neurol., [2]Psychiatry, [3]Radiology, [4]Pediatrics, [5]Neurosci., Washington Univ. Sch. of Med., Saint Louis, MO; [6]Ctr. for Excellence for Res. on War Veterans, Waco, TX; [7]Ctr. for Vital Longevity, Univ. of Texas Dallas, Dallas, TX; [8]Psychological and Brain Sci., Washington Univ. in St. Louis, Saint Louis, MO; [9]Psychology and Neurosci., Baylor Univ., Waco, TX

Abstract: Numerous investigations into resting state functional MRI (fMRI) have provided converging group-level descriptions of functional brain organization at the systems levels. Recent work from our lab has revealed that functional systems defined in one highly sampled individual contain distinct topological features that deviate from the group-level systems description (Laumann et al., 2015. Neuron). We describe these features as *topological variants*. Here, we extend our investigation of individual differences in functional brain organization to a new data set of 10 highly sampled young adults for whom 5 hours of resting state fMRI were collected (see Nelson et. al.). Analyzing 9 of the subjects with sufficient high-quality data we find that topological variants are present in all individuals. Further, we demonstrate that these variants are reliably observed in each subject across ten independent 30-minute fMRI sessions. Topological variants occur commonly in certain regions of cortex, for instance near the frontal operculum and the temporo-parietal junction, and rarely occur in other regions, such as primary sensorimotor, auditory, and posterior cingulate cortex. Moreover, we show that there are stereotypic patterns of topological variants, i.e. sets of variants that tend to co-occur across individuals. However, the functional systems to which variants are assigned appear to vary across individuals, even for variants that occur in the same anatomical location. Our preliminary findings suggest that topological variants are reliable components of adult functional brain organization at the individual-level. Future work in larger groups of subjects will help determine the full distribution of topological variants across healthy individuals.

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Poster

745. Human Cognition: Individual Differences I

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Support: JS McDonnell Foundation (#22002082)

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Title: Network markers of individual variability in the human functional connectome.

Authors: C. PENA[1], A. AVENA-KOENIGSBERGER[1], J. SEPULCRE[3], *O. SPORNS[2]; [2]Psychological and Brain Sci., [1]Indiana Univ., Bloomington, IN; [3]Harvard Univ., Boston, MA

Abstract: A repertoire of individually variable patterns of functional connectivity (FC) underpins a broad set of individual patterns of human behavior and cognition. However, to date most neuroimaging studies have focused on consistent patterns in group-averaged functional connectivity (FC) and related graph measures. Lately, two new avenues have appeared in the field of functional connectomics. First, several studies have examined markers of individual variability using functional connectivity. Second, a different set of studies revealed significant dynamical variations within the same session challenging the static view of functional connectivity. In the present study we used both static and dynamical functional connectivity approaches to study the individual functional connectome. We aimed for two main goals. First, we set out to determine the minimal number of brain regions required to identify individual subjects (the minimal FC fingerprint). Second, we characterized different profiles of the intra-inter network functional connectivity the fingerprints across time. Forty subjects underwent two sessions of resting fMRI separated one month apart. Using standard preprocessing steps FC was measured for each subject as the Pearson pair-wise correlation of regional time series. Each matrix was reshaped into a vector containing the individual pattern of connectivity for each session. We then assessed the similarity across session computing the similarity amongst those vectors. These analyses were conducted under a wide range of parcellations (~48-2000 nodes) as well as different voxel resolution (2-4-8 mm). Finally, we implemented a simulated annealing optimization algorithm to determine the minimal FC fingerprint in seven resting state networks. In addition to static FC analysis, a temporal characterization was performed using windowed patterns. We found that the frontoparietal network contains network markers that allow identification of individuals with 100% accuracy, with lower levels of accuracy found in other networks. We also found that this fingerprint was expressed at different levels across time. Our study could be useful for characterizing individual differences in brain networks and in future applications in personalized connectomics.

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Poster

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Topic: H.02. Human Cognition and Behavior

Title: When the going gets tough, the low cholinergic stop going: Cholinergic genetic variation and right prefrontal activation, signal detection, and real-world distraction

Authors: ***A. CAPLE**[1], Z. LIN[1], A. BERRY[2], R. D. BLAKELY[3], M. SARTER[1], C. LUSTIG[1]; [1]Psychology, Univ. of Michigan, Ann Arbor, MI; [2]Life Sci. Div., Lawrence Berkeley Natl. Lab., Berkeley, CA; [3]Dept. of Biomed. Sci., Florida Atlantic Univ., Jupiter, FL

Abstract: Right middle/inferior frontal gyrus activation has been linked to "attentional effort", the motivated recruitment of attention and cognitive control. Parallel rodent and human studies suggest that cholinergic inputs to right PFC make a critical contribution to the increased activation seen in human fMRI studies during demands on attention (see Lustig & Sarter, in press, for a recent view). We previously reported that a genetic polymorphism thought to reduce cholinergic efficiency (Ile89Val variant of the presynaptic choline transporter gene, rs1013940) was associated with reduced right PFC activation during a perceptual-attentional challenge (Berry et al., 2015). Here we report secondary analyses more closely examining the relationship of right PFC activation and connectivity to behavior. In contrast to controls, Ile89Val participants failed to activate right PFC in response to a perceptual-attentional challenge. Moreover, in a sizable proportion of participants (30%), the right PFC not only failed to activate but activity in this region decreased below levels observed in the absence of such a challenge. For Ile89Val participants, reduced or reversed right PFC activation was associated with reduced performance, specifically an increase in false alarms and in response-time variability during correct rejections. Reduced right PFC activation during the challenge condition also correlated with self-report measures of real-world distractibility. Together, these results suggest that reduced cholinergic function and right PFC activation lead to a decrease in attentional effort in challenging conditions, and instead an increased reliance on bottom-up salience.

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Poster

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Title: Individual variability and connectivity dynamics in modular organization of human cortical functional networks

Authors: *M. FUKUSHIMA[1], R. F. BETZEL[2,1], Y. HE[1,3], M. A. DE REUS[4], M. P. VAN DEN HEUVEL[4], X.-N. ZUO[3], O. SPORNS[1,5];
[1]Dept. of Psychological and Brain Sci., Indiana Univ., Bloomington, IN; [2]Dept. of Bioengineering, Univ. of Pennsylvania, Philadelphia, PA; [3]Inst. of Psychology, Chinese Acad. of Sci., Beijing, China; [4]Brain Ctr. Rudolf Magnus, Univ. Med. Ctr. Utrecht, Utrecht, Netherlands; [5]Indiana Univ. Network Sci. Inst., Bloomington, IN

Abstract: There is growing evidence for modules in anatomical networks of neural populations and brain regions. Modular organization is also found in functional networks, in which connectivity is defined based on statistical dependencies between neuronal or regional activities. In such functional networks derived from human functional magnetic resonance imaging, individual variability in modularity exhibits possible relationships with variations in demographics and behavioral performance. In parallel, recent studies have demonstrated that functional networks during resting state vary on a time scale of tens of seconds. However, little is known about fluctuations of modularity in time-varying networks, as well as their relation to individual variations in modularity measured over longer time scales. In the present study, we relate individual variations and dynamic fluctuations of modularity and investigate connectivity patterns during periods of high and low modularity. Data were derived from a part of the

enhanced Nathan Kline Institute-Rockland Sample (NKI: n = 80, 18-30 years) and the Human Connectome Project (HCP: n = 84, 22-35 years). Time series were averaged within each cortical region in a functional (NKI: 113 regions) and an anatomical parcellation (HCP: 114 regions). The Pearson correlation was used as a metric of functional connectivity. Time-resolved functional connectivity was estimated using a sliding-window approach. Modularity was quantified using a quality function maximized by a community detection algorithm. Periods of high and low modularity were determined based on null distributions of fluctuations in modularity amplitude. We found that individual differences in modularity across subjects persisted across multiple resting-state sessions. In subjects exhibiting high modularity over longer time scales, time-resolved functional connectivity showed highly modular network structure more frequently. Time-resolved functional networks observed during periods of high modularity exhibited greater similarity to each other, compared to periods of low modularity. The high/low modular state centroid exhibited lower/higher similarity to structural connectivity, respectively, indicating that connectivity patterns averaged within periods of low modularity more closely reflect connectivity patterns of anatomical networks. Taken together, these results suggest that (long time scale) individual variations and (short time scale) fluctuations of modularity are interrelated, wherein the dynamics of modularity emerge from fluctuations around relatively uniform to structurally-based variable connectivity patterns.

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Poster

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Title: Deep dynamic phenotyping of the individual: Estimating within-subject cortical thickness to 10μm precision using cluster scanning

Authors: *J. A. NIELSEN[1,2], B. R. FISCHL[2,3], R. L. BUCKNER[1,2,3];
[1]Harvard Univ., Cambridge, MA; [2]Massachusetts Gen. Hosp., Boston, MA; [3]Harvard Med. Sch., Boston, MA

Abstract: Most studies of human brain anatomy have identified differences and patterns across groups of individuals. They have yielded important insights into the general properties of brain morphometry, trajectories through development, and group differences. However, changes that occur within individuals throughout development have not been studied and are typically considered too small to detect (e.g., developmental changes in cortical thickness are ~40μm/yr). The primary goal of the present work is to explore a method for detecting subtle changes in brain morphometry within an individual over a short period of time. The central idea is to take advantage of the extensive scanning we have recently conducted in four individuals to examine the limits of anatomical estimation. During each of 24 sessions per participant, a T1-weighted ME-MPRAGE image was acquired (Siemens 3T Prisma, TR=2.2s, TI=1.1s, TE=1.57ms to 7.03ms, FA=7°, vox=1.2mm, FOV=230mm). The structural images were analyzed using FreeSurfer 4.5.0. Error estimates were calculated by comparing estimates from independent data sets acquired within the same individual on separate days. Estimates of brain morphometry based on individual structural images resulted in typical error terms. As examples, the mean error in cortical thickness for a region of middle frontal gyrus was ~50μm (2.0%) and in volume for the thalamus was ~160mm^3 (2.0%). However, when we implemented cluster scanning (i.e., the acquisition of multiple images over a short period of time followed by averaging the estimates), the error decreased dramatically. The error in middle frontal gyrus decreased to ~12μm (0.5%) when averaging the estimates from 12 images (~30min total scanning); the error in thalamic volume decreased to ~40mm^3 (0.5%). Global cortical thickness could be estimated with a precision of ~5μm. We believe the high level of precision results from combining three factors: (1) sampling the cortical ribbon over thousands of voxels per image, (2) minimizing anatomical blurring by acquiring brief individual acquisitions, and (3) sqrt n signal averaging afforded by combing multiple estimates. Cluster scanning may be a fruitful method for detecting subtle changes in brain anatomy within an individual. The method may be particularly useful in the context of development (e.g., thinning of the cortical sheet during adolescence) or disease (e.g., accelerated atrophy due to dementia).

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Poster

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Title: Unique contributions of cortical brain characteristics to fluid intelligence

Authors: *C. FRAENZ, C. SCHLUTER, P. FRIEDRICH, O. GUNTURKUN, E. GENC; Dept. of Biopsychology, Ruhr-University, Bochum, Germany

Abstract: Previous research demonstrated that variations of diverse brain characteristics like gray and white matter volume predict differences in fluid intelligence. Studies employing graph theory to analyze the brain's structural and functional network connectivity found that individuals with pronounced fluid intelligence show higher values with regard to global efficiency. It is reasonable to assume that the aforementioned brain characteristics are correlated with each other. Therefore, we used an integrative approach to identify the unique contributions from each of the different brain characteristics in predicting fluid intelligence.
We measured fluid intelligence in a sample of 120 healthy participants by conducting a non-verbal intelligence test (BOMAT). In addition to that, we used MRI, DTI and resting-state fMRI measurements to examine the following brain characteristics: gray matter volume (GMV), white matter volume (WMV), structural and functional network connectivity (NET DTI & NET REST) as indexed by global efficiency, a measure yielded by graph theory. Using multiple regression analysis, we searched for unique contributions from each of the different brain characteristics in predicting fluid intelligence.
As expected, statistical analyses showed that GMV ($r = .34$, $p < .001$), WMV ($r = .19$, $p = .03$), NET DTI ($r = .23$, $p = .01$) and NET REST ($r = .28$, $p = .002$) are significantly correlated with fluid intelligence. Importantly, we found significant correlations between GMV and all other brain characteristics as well ($r = .27$ - $.78$, $p < .01$). Therefore, we performed a multiple regression analysis with GMV, WMV, NET DTI and NET REST as the independent variables and fluid intelligence as the dependent variable. Surprisingly, only GMV and NET REST provided unique contributions to the prediction of fluid intelligence (GMV, $ß = .36$, $p = .03$; NET REST, $ß = .20$, $p = .03$; other brain characteristics, $p > .31$). The pattern remained stable when controlling for the effects of age and gender.
In the past, brain characteristics predicting fluid intelligence were investigated independently of one another. Our work features an integrative approach that highlights the important interplay of such brain characteristics. Our results show that the influence of certain brain characteristics on fluid intelligence like WMV and NET DTI is scaled down heavily when examined in

combination with other brain characteristics like GMV and NET REST. Therefore, we conclude that gray matter volume and functional connectivity play major roles in the neural composition of fluid intelligence, whereas white matter volume and structural connectivity appear to have no unique contribution.

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Poster

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Title: High-fidelity mapping of individual human brain organization and function

Authors: ***S. M. NELSON**[1,2,3], T. O. LAUMANN[4], A. W. GILMORE[5], J. J. BERG[5], D. J. GREENE[6,7], E. M. GORDON[1,2], M. ORTEGA[4], A. NGUYEN[4], C. HOYT-DRAZEN[4], R. S. COALSON[4,6], K. B. MCDERMOTT[5,6], J. S. SHIMONY[6], A. Z. SNYDER[4,6], B. L. SCHLAGGAR[4,6,8,7,9], S. E. PETERSEN[4,6,5,9], N. U. F. DOSENBACH[4];
[1]Ctr. of Excellence for Res. on War Veterans, Waco, TX; [2]Ctr. for Vital Longevity, Univ. of Texas - Dallas, Dallas, TX; [3]Psychology and Neurosci., Baylor Univ., Waco, TX; [4]Neurol., [5]Psychological and Brain Sci., [6]Radiology, [7]Psychiatry, [8]Pediatrics, [9]Anat. & Neurobio., Washington Univ. in St. Louis, Saint Louis, MO

Abstract: Until recently, functional neuroimaging research has focused almost exclusively on studies of groups, not individuals, largely because the noise properties of the blood-oxygen level dependent (BOLD) signal require large amounts of data. Thus, the majority of functional neuroimaging studies have produced functional maps of an important research construct: the

group-average brain. While we have learned a great deal about the central tendencies of human brain organization from studies of group-averaged BOLD data, knowing more about the functional network organization of individual humans will be important in considering how neuroscience can contribute to the treatment of neuropsychiatric disorders. Laumann *et al.* (2015) produced the first high-fidelity individual functional network map by analyzing many hours of resting state functional connectivity MRI (rs-fcMRI) data collected on a single individual. They validated their single subject network map by annotating it with task-based fMRI data from the same individual and by comparing it to an existing group-averaged functional network map. This work provided a proof-of-principle for functional neuroimaging in individuals. Here, we present 10 subsequent high-fidelity individual network maps (5 F) based on 5 hours of rs-fcMRI, 10 hours of task-based fMRI and 5 hours of structural MRI data, per subject. This study verified and generalized the findings reported by Laumann et al. First, we found that the functional connectivity matrix of each individual was relatively stable across scanning sessions. We also observed that the functional organization of each individual brain was significantly more complex and detail-rich than the group-average map, even in our demographically homogenous sample of 10 healthy young adults (age 24-34). Using this subject-specific approach, we were able to identify new common network pieces, obscured in group-averaged data, most likely because of their smaller size. For example, in individuals we found that small regions in bilateral frontal cortex belong to the visual network. Although the focus here is on newly discovered network pieces common to all, similar analyses using the same set of data are investigating inter-individual variants in topology (see Seitzman *et al*.).High-fidelity functional network mapping will enable us to study single individuals with rare brain lesions, disorders or cognitive skills that might hold important clues about fundamental principles of human brain organization. Repeatedly scanning individuals also points out a straightforward path towards developing sensitive and specific functional neurodiagnostics.

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Poster

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Title: The relationship between axon density, myelination and microstructure in the human corpus callosum

Authors: *E. GENC, C. FRAENZ, P. FRIEDRICH, C. SCHLÜTER, S. OCKLENBURG, O. GUNTURKUN;
Ruhr Univ. Bochum, Bochum, Germany

Abstract: Signals from sensory half-fields are processed separately in the two hemispheres of our brain but give rise to a coherent percept due to interhemispheric integration. Previous research indicates that functional communication between the two hemispheres is modulated by the microstructure of the corpus callosum (CC). DTI fractional anisotropy (FA) can be considered as the most commonly used measure to describe the tissue microstructure of human white matter. However, physiological factors determining this anisotropy are not fully understood. Since CC fibers are strictly oriented in the left-right direction and do not show any crossings, this structure provides an excellent opportunity to study the influence of axon density and myelination on FA.
We acquired brain images from 218 healthy participants using four different MRI protocols. A high-resolution structural image was used to determine anatomical landmarks as seed regions for fiber-tractography. Further, a DTI measurement in combination with probabilistic fiber tracking was used to precisely define twelve different CC segments interconnecting homotopic regions of visual, parietal, temporal, somatomotor and frontal areas. Average FA values were computed for each segment. In addition to this, multi-shell NODDI and GRASE protocols were used in order to examine the axon density and myelination of the respective CC segments.
Statistical analyses showed a posterior-to-anterior gradient for axon density and myelination of callosal fibers. Further, we performed PCA analyses for each of the three white matter properties and found that the FA, axon density and myelination values of all CC segments could always be grouped into three components. Visual and parietal segments highly loaded on the first component, temporal and somatomotor segments on the second component and frontal segments on the third component. Additionally, we correlated the FA, axon density and myelination values of each CC segment with each other and found a constant positive association between the three white matter properties ($r = .17 - .51$, $p < .01$). Finally, we performed multiple-regression analyses to demonstrate that the average FA value of a given CC segment can be predicted more precisely by its axon density ($ß = .29 - .48$, $p < .01$) than its myelination ($ß = .01 - .21$, $p > .07$). Our study is the first to examine the *in vivo* distribution of axon density and myelination of human callosal fibers. Importantly, we demonstrated that in a highly coherent white matter structure like the CC axon density had a stronger contribution on anisotropic diffusion than myelination.

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Poster

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Title: Cognitive control network global connectivity is related to the mental health of healthy individuals

Authors: *D. H. SCHULTZ, T. ITO, L. I. SOLOMYAK, R. H. CHEN, R. D. MILL, K. R. KULKARNI, M. W. COLE;
CMBN, Rutgers-Newark, Newark, NJ

Abstract: We all vary in our mental health, even among "healthy" non-diagnosed individuals. To better understand the large-scale network mechanisms underlying this variability in mental health we examined the relationship between mental health symptoms and functional connectivity patterns in cognitive control systems. The frontoparietal cognitive control system consists of flexible hubs that can regulate distributed systems depending on current goals and dysfunction in the frontoparietal network (FPN) has been identified in a variety of psychiatric disorders. Alterations in FPN connectivity may influence mental health by disrupting the ability to regulate symptoms in a goal-directed manner. This suggests that the FPN may play an important role in the promotion and maintenance of mental health. We tested the hypothesis that disruptions in FPN connectivity are related to mental health symptoms. Previous studies have found differences in activity and connectivity of brain networks between healthy controls and individuals diagnosed with psychiatric disorders. Importantly, we examined this association in a non-medicated, non-diagnosed population of young adults. We collected data from 73 young adults, with participants completing the Center for Epidemiologic Studies Depression Scale Revised (CESD-R) and a resting-state fMRI scan. Functional connectivity (FC) was calculated between a standard set of functionally defined regions throughout the brain. Global brain connectivity (GBC) was calculated as the mean of FC values for each node (excluding nodes assigned to the same functional network). We found a significant negative correlation between GBC in the FPN and depression symptoms. This suggests that decreased connectivity between the FPN and the rest of the brain is related to increased symptoms of depression. In order to determine which specific connections were influenced we first used the Human Connectome Project as a discovery dataset. We correlated depression and anxiety symptoms with rest FC and identified FPN connections to probe in our independent data. Within these FPN connections of interest we found significant negative correlations between FPN-visual and FPN-sensorimotor connections and depression symptoms. Together these results suggest that decreased global FPN

connectivity is associated with increased mental health symptoms, and specific connections between the FPN and portions of the visual and sensorimotor networks contribute to this relationship. This data supports the hypothesis that global FPN connectivity contributes to the regulation of mental health symptoms.

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Poster

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Title: Deep dynamic phenotyping of the individual: Exploring brain state dynamics over multiple timescales

Authors: *R. HUTCHISON, III[1,2], R. M. BRAGA[2,3,4], J. T. BAKER[5,6,3], R. L. BUCKNER[2,3,6]; [1]Neurosci., [2]Harvard Univ., Cambridge, MA; [3]Massachusetts Gen. Hosp., Boston, MA; [4]Imperial Col. London, London, United Kingdom; [5]McLean Hosp., Belmont, MA; [6]Harvard Med. Sch., Boston, MA

Abstract: Most studies of human brain function have focused on trait estimates measured at one moment in time or a few longitudinal estimates spaced across extended delays. Dynamic fluctuations over days, weeks, and months have not been well studied despite a range of behavioral effects fluctuating over these timescales. The present work focuses on in-depth, longitudinal assessment of behavioral, physiological, and brain dynamics that are tailored to the specific network topography and temporal features of an individual. A primary goal is to explore the variability of evoked activity and functional coupling that occurs on multiple time scales -

spanning minutes to months - and their relationship to physiological and behavioral measures including autonomic arousal, mood, activity levels, and sleep quality. In 4 female young adults, a brief core battery of functional tasks was acquired on separate days simultaneously with physiological measures (heart rate, respiration, GSR) and eyetracking (pupil size, position, eye closures). Visuomotor (120s), face matching (120s), rule switching (108s), 2-back (108s), mental rotation (108s) microtasks and a passive fixation rest (422s) task were collected. Scanning was spaced over four months within two dense sampling periods (each period acquired 12 sessions spread across 28 days). Stimuli were not repeated across sessions. Across all tasks, simultaneous multi-slice sequences were used (Siemens Prisma, SMS=5, TE = 32.6ms, TR=1.0s, vox=2.4mm). Task-based and functional connectivity analyses revealed both stable and unstable features of brain activity patterns at a regional and network level within a task type across sessions. Dynamic functional connectivity analysis showed unique day-to-day signatures of state expression that were related to the differences in the static maps. The variability in magnitude estimates, frequency differences in functional configuration expression, and shifts in the temporal ordering of brain patterns will be linked to ongoing passive and active data collection. The focus on dense longitudinal study of the individual may provide a foundation to better understand brain states and network interactions that change over time including fluctuations in mood, addiction, and responses to stressful life events.

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Poster

745. Human Cognition: Individual Differences I

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Title: Deep dynamic phenotyping of the individual: mapping idiosyncratic features of network and regional architecture

Authors: *R. M. BRAGA[1,2,3], R. HUTCHISON[1], H. LIU[3,4], R. L. BUCKNER[1,3,4];
[1]Ctr. for Brain Sci., Harvard Univ., Cambridge, MA; [2]Imperial Col. London, London, United Kingdom; [3]Athinoula A. Martinos Ctr. for Biomed. Imaging, Massachusetts Gen. Hosp., Charlestown, MA; [4]Harvard Med. Sch., Boston, MA

Abstract: There is growing interest in mapping the detailed functional architecture of individuals for clinical purposes (e.g., presurgical planning) as well as for discovery. In particular, while group-averaged data have been a tremendous engine for discovery of broad organizational properties, between-subject blurring and dependence on central tendencies may obscure organizational details. Here we analyzed the detailed organization of within-subject data collected over many sessions. In addition to 24 core sessions that collected a limited set of functional tasks, four extended sessions were acquired for in depth localization of functional regions. These sessions included many different tasks selected to probe domains that preferentially activate distinct brain regions including: motor topography, retinotopy, sentence reading, word classification, working memory (n-back), judgments of remembering, visuomotor response, emotional face perception, theory-of-mind, social movie viewing, mental rotation, and task switching. Extensive resting-state (passive fixation) data were also available for network estimation. The resulting maps were found to be reliable estimates of each individual's task active and resting-state networks. When individual participant maps were compared with group-averaged maps of the same task contrasts, differences in functional organization were clearly observed that could not be fully explained by registration or anatomical differences. In addition, large-scale intrinsic connectivity networks were found to contain heterogeneous subregions at the individual subject level, with some group-wise canonical networks being divisible into two or more networks based on functional connectivity. One prominent example occurs at or near the posterior cingulate. These results provide insight into how an individual's functional organization differs from that derived from group-average approaches to brain mapping. Parallel ongoing work is applying these insights to understand how an individual's functional organization varies day-to-day in healthy subjects, as well as individuals under stress and suffering from mood disorders.

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Poster

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Topic: H.02. Human Cognition and Behavior

Support: NSF Grant BCS 1228595

Title: Distinct neural network dynamics are involved in three aspects of cognitive flexibility, determined at the trial level

Authors: *K. B. STEELE[1], S. MOLHOLM[1,2], J. J. FOXE[1,2,3];
[1]Neurosci., [2]Pediatrics, Albert Einstein Col. of Med., Bronx, NY; [3]Inst. for Neuromedicine and the Dept. of Neurosci., Univ. of Rochester, Rochester, NY

Abstract: An integral aspect of cognitive control is the ability to flexibility switch our attention between competing streams of information, and to do so seamlessly. This seemingly effortless skill belies the multitude of necessary processing steps along with the many brain regions involved. This results in a small but consistent switch cost, observable as delays in response time and as extra activity in the frontoparietal network. The switch cost is influenced by the presence of distracting, incongruent stimuli and whether the current task requires the same or a different motor response to the last. We sought - using high-density electroencephalography and blind source separation - to demonstrate that individuals are differentially affected by switching, incongruency, and response-switching, and that these differences are associated with functional variations in network processing. Ten neurologically-normal adults completed four separate task-switching sessions while being recorded with a 256-electrode EEG cap. Reaction time data demonstrated that faster responders were also more accurate, indicating there may be a spectrum of network efficiency. Surprisingly, not everyone exhibited a switch cost, some only had an incongruent cost and/or response-switch cost. This indicated there may be different aspects of the neural network causing the different types of behavioral delays. Independent component analysis (ICA) pulled out the activity arising from task-responsive neural sources. Across all individuals, frontoparietal regions were the majority of regions involved, such as the superior parietal cortex, inferior parietal cortex, middle frontal gyrus, superior temporal gyrus, and cingulate cortex. Using a causal inference algorithm to estimate the Markov equivalence class, which takes into account the network's activity as a whole, we determined that task-switching activity initiates in the middle frontal gyrus and left superior parietal cortex, passes it onto cingulate cortex, right superior parietal cortex, and finishes in middle frontal again as well as precentral (i.e. motor) cortex. Overall, switch costs were more strongly associated with parietal activity while incongruent delays were more associated with bilateral insular activity whereas response-switch costs were associated with less activity in the anterior cingulate. To conclude, individuals do not all have switch costs and are predisposed to be affected by different sources of conflict. This is most likely arising from differences in the temporal dynamics of the frontoparietal regions recruited for processing.

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Poster

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Topic: H.02. Human Cognition and Behavior

Support: AFOSR FA9550-12-10388

Title: Neural entrainment to the beat: The "missing pulse" phenomenon

Authors: *E. W. LARGE[1], E. M. ZION GOLUMBIC[2], C. E. SCHROEDER[3], D. POEPPEL[4]; [1]Dept. of Psychology, Univ. of Connecticut Dept. of Psychology, Storrs Mansfield, CT; [2]Gonda Brain Res. Ctr., Bar Ilan Univ., Tel Aviv, Israel; [3]Dept. of Psychiatry, Columbia Univ. Med. Ctr., New York, NY; [4]Dept. of Psychology, New York Univ., New York, NY

Abstract: Most humans have a near-automatic inclination to tap, clap or move to the beat of music. The perception of a periodic pulse in a highly complex musical rhythm is remarkable as it requires neural abstraction of the temporal structure of the stimulus. It has been suggested that nonlinear interactions in neural networks give rise to cortical oscillations at the beat frequency, and that entrained oscillation gives rise to the percept of pulse. Here we tested this "neural resonance theory" using MEG recordings as individuals listened to rhythmic stimuli. Participants listened to 30-second long sequences of complex syncopated drum sounds designed so that they contain no energy at the pulse frequency. We analyzed the spectrum of the neural activity while listening and compared it to the spectrum of the stimuli. We found neural resonance in auditory cortex at the pulse frequency, and showed phase locking to the missing pulse, despite the fact that this frequency was absent from the stimulus itself. Moreover, the strength of this response correlated with individuals' ability to tap the pulse of these stimuli, as tested in a follow-up session. These findings demonstrate that neural activity at the pulse-frequency in auditory cortex is internally generated rather than stimulus-driven, supporting the neural resonance theory. Critically, our results suggest that neural entrainment to the pulse is a fundamental dynamical mechanism underlying musical skill.

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Poster

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Topic: H.02. Human Cognition and Behavior

Support: BC LEEF, CFI, NSERC, CIHR

Title: The alpha-theta-gamma (ATG) switch - a neurophysiological and conceptual perspective

Authors: *U. RIBARY[1,2], S. M. DOESBURG[1], L. M. WARD[2];
[1]Simon Fraser Univ. (SFU), Burnaby, BC, Canada; [2]Univ. of British Columbia (UBC), Vancouver, BC, Canada

Abstract: Sensory, motor and cognitive functions are associated with oscillatory changes within task-relevant cortical regions, as well as alterations in functional and effective connectivity between those regions associated with neuronal synchronization. Magnetoencephalography (MEG) and Electroencephalography (EEG) have shown that brain dynamics within and between regions often differ markedly across frequencies, and that brain rhythms often interact across widely separated frequency ranges. It has further been reported that cross-frequency interactions may play an important role in local processing within thalamus and neocortex, as well as information transfer between subcortical and cortico-cortical brain regions. Strong commonalities in rhythmic network properties have been observed across recording techniques and task demands, but strong neuroscientific theories to situate such observations within a unified framework with direct relevance to explain neuropathologies remain scarce.
A comprehensive review into the animal and human literature indicates a further thinking beyond synchrony and connectivity and the readiness for more hypothesis-driven research and modeling toward unified principles of thalamo-cortical processing. We further introduced such a possible framework: "The ATG switch" *(Doesburg et al. 2015)*. We probed this neurophysiological framework to explain how coordinated cross-frequency and interregional oscillatory cortical dynamics may underlie typical and atypical brain activation, and the formation of distributed functional ensembles supporting cortical networks underpinning sensation and cognition. We also discussed evidence that alpha-theta-gamma dynamics emerging from thalamocortical interactions may be implicated and disrupted in numerous neurological and neuropsychiatric conditions.
We present a conceptual overview of this framework as a novel plausible mechanism to integrating local and large-scale thalamocortical brain networks and discuss its alterations in clinical pathologies.
Ref: Doesburg, Ward and Ribary. Curr. Trends Neurol., 9, 1-12, 2015.

Disclosures: **U. Ribary:** None. **S.M. Doesburg:** None. **L.M. Ward:** None.

Poster

746. Human Cognition: Temporal Processing II

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Program#/Poster#: 746.04/KKK42

Topic: H.02. Human Cognition and Behavior

Title: Hazard rate modulates reaction time to auditory and visual stimuli with complex probabilistic structure

Authors: *M. GRABENHORST, G. MICHALAREAS, C. ABEL, V. DEHMELT, D. POEPPEL;
Neurosci., Max Planck Inst. (MPIEA), Frankfurt, Germany

Abstract: Anticipating the timing of salient events is required for successful interaction with the environment. Temporal cues embedded in visual information are encoded in two processes: representing elapsed time and calculating the probability of an event over time - a computation termed the hazard rate (HR) (Janssen and Shadlen, 2005). If such a computation is fundamental and generic, it should be visible across sensory modalities. Our experimental design is based on the rationale that knowledge of temporal stimulus probability (i.e. HR) enables allocation of sensory resources which modulates reaction time (RT). In a series of psychophysical experiments participants' RTs to auditory and visual stimuli were recorded. The temporal trial structure was the same in auditory and visual blocks and consisted of 'ready', 'set' and 'go' cues. Time between 'set' and 'go' ('go' time) was a random variable drawn from either a uni- or bimodal probability distribution (PD) which was fixed throughout a block. The HRs derived from the two PDs were not correlated with each other and were therefore hypothesized to display individual patterns of RT modulation. We found that irrespective of the presented PD, mean RT was significantly faster in auditory blocks, replicating typical findings in psychophysics (Welford, 1980). In unimodal blocks RT decreased with longer 'go' times (regression analysis). In bimodal blocks RTs were fastest for 'go' times drawn from the second mode. These findings replicate results from a similar visual task in macaque (Janssen et al 2005) and humans (Bueti et al, 2010). To analyse the relationship between HR and RT we fitted a regression model: The HR appropriate for the respective PD modulated RT in both visual and auditory conditions. This indicates that participants learned not only the timespan of possible 'go' times but also the underlying probabilistic structure encoded in the HR. Our findings suggest that the HR may be a basic computation in probability learning in both sensory modalities.

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Poster

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Topic: H.02. Human Cognition and Behavior

Support: CIHR Operating Grant MOP 115043

Title: Auditory rhythm entrainment relates to prediction of pitch: the role of beta oscillation

Authors: *L. J. TRAINOR, A. CHANG, K. CLAYWORTH, D. BOSNYAK;
McMaster Univ., Hamilton, ON, Canada

Abstract: Neural activity in auditory cortex predicts upcoming dynamic information prior to an event's occurrence, including predicting when (time) an event will occur and what (e.g., pitch) will occur. These predictions facilitate perceptual processing. Previous studies have shown that the induced power of beta oscillations (15 - 25 Hz) in auditory cortex reflects the characteristic of time prediction. Specifically, beta power fluctuations entrain to the rate of a presented isochronous tone sequence. However, it is unclear whether the beta oscillations only predict the timing of auditory information, or whether they also reflect predictions for the contents of auditory information, such as pitch. Our first study used rhythmic auditory oddball sequences to show that induced beta power entrainment is modulated after the presentation of an unexpected pitch change, even when the change is presented at a predicted time point. This suggests beta oscillatory activity is modulated by the prediction error for pitch in additional to entraining to rhythm. The second study employed rhythmic auditory oddball sequences with predictable versus unpredictable pitch changes (equating change probability) to show that beta oscillation is modulated by the prediction to the deviant pitch prior to its onset: (1) larger beta power entrainment depth prior to deviant pitches was found in the predictable than unpredictable case, (2) predictable deviant pitches evoked smaller P3a amplitude, a late event-related potential reflecting exogenous attentional orienting to deviance, than the unpredictable deviant pitches, and (3) trial-to-trial analyses showed that beta power entrainment depth was negatively correlated with P3a amplitude only in the predictable context. Together, our findings to date suggest that in addition to predicting when an event will occur, beta activities also reflect the prediction of what will occur.

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Poster

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Topic: H.02. Human Cognition and Behavior

Support: U.S. Army Research Office Grant W911NF-09-0001

Title: Intentional binding with sustained visual feedback: A paradigm compatible with chronometric TMS

Authors: *A. D. SHAPIRO[1], L. J. VOLZ[2,3,1], T. G. LEE[4], S. T. GRAFTON[1];
[1]Psychological and Brain Sci., [2]SAGE Ctr. for the Study of the Mind, [3]Inst. for Collaborative Biotechnologies, Univ. of California, Santa Barbara, Santa Barbara, CA; [4]Psychology, Univ. of Michigan, Ann Arbor, MI

Abstract: The subjective sense of agency (SoA) we experience over our actions and their consequences may support goal-directed behavior by reinforcing the causal relationship between intentions, actions, and predicted outcomes. One marker of causality is the temporal proximity of two events. On this logic, the intentional binding phenomenon, in which voluntary actions and their intended consequences are perceived as shifted towards each other in time, is thought to be an implicit measure of the SoA. Ongoing debate concerns whether the SoA is primarily a prospective phenomenon driven by predictive motor control processes or a retrospective phenomenon driven by inferences made after the perception of sensory feedback. Relevant work on the functional anatomy supporting these accounts implicates the pre-supplementary motor area (preSMA) and inferior parietal regions including the angular gyrus (AG). Yet this evidence relied on neuroimaging and offline neurostimulation methods that lack sufficient temporal resolution to inspect the interplay between these regions at sub-trial timescales. Chronometric TMS promises the appropriate specificity for such investigations. However, the audible clicks emitted by TMS discharge and targeting sensorimotor regions would confound the brief (100ms) auditory and tactile stimuli traditionally used in intentional binding. Visual stimuli have been sparsely used as sensory feedback in this paradigm and the relative effects of visual feedback, concurrent visual + auditory feedback, and feedback duration had not been explored. In Experiments 1-3 we measured intentional binding of voluntary button presses paired with sustained visual feedback (1.5-2.5s) relative to other feedback stimuli. Intentional binding with sustained visual feedback did not differ significantly from binding with brief auditory feedback (Exp 1, n=21, p=.22) or combined auditory + visual feedback (Exp 3, n=20, p=.62). However, binding was significantly diminished with brief visual feedback relative to sustained visual feedback (Exp 2, n=22, p=.01). This may be due to the flash-lag effect wherein a flashed visual stimulus appears to lag behind a moving stimulus on which it was superimposed, such as the

revolving clock hand used in this study. Taken together, Experiments 1-3 validated that sustained, but not brief, visual feedback evokes intentional binding. Ongoing research investigates the effects of chronometric TMS to preSMA and AG during time windows for predictive processing and retrospective processing during voluntary actions, allowing us to inspect the dynamic contributions of these regions to the SoA in intentional binding.

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Poster

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Topic: H.02. Human Cognition and Behavior

Support: Gertrude F. Ribble Trust Mini Grant

Title: Eeg and reaction time profiling in novice meditators

Authors: *S. S. JOSHI[1], A. PATEL[1], A. AGARWAL[2], B. F. O'HARA[1];
[1]Biol., Univ. of Kentucky, Lexington, KY; [2]Signal Solutions LLC, Lexington, KY

Abstract: Previous studies on meditation have found EEG spectral properties and other features suggesting that it may be a state that is distinct from sleep and wake. A number of benefits have been claimed by practitioners of meditation which have gained increasing attention over the past several decades, with an increase in the number of scientifically controlled studies in recent years. In a previous study conducted by our group, a short term performance improvement was observed on a psychomotor vigilance task (PVT) in both experienced and novice meditators after completing 40 minutes of meditation. In the PVT, mean reaction time (RT) is typically calculated based on the responses of subjects to a stimulus. In this current study, a similar experiment was conducted on a cohort of 30 subjects wherein the reaction time assessments were done before and after 20 minutes of meditation. Mean RT measurements were done using the standard PVT-192 monitor. While subjects were meditating, EEG recordings were done using Emotiv EPOC wireless EEG systems. All subjects were in the age range of 18-55 years, and were recruited at the University of Kentucky through flyers and word of mouth. In a pairwise t-test, significant improvement was seen in post meditation PVT. Based on the EEG recordings, we intend to prepare models for brain activity in novice meditators during meditation, correlating spectral power, and other EEG features, to performance measures. Data and results from multiple on-going studies will be presented.

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Poster

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Topic: H.02. Human Cognition and Behavior

Support: JSPS Research Fellowships for Young Scientists

Title: Duration adaptation improves time sensitivity

Authors: ***M. J. HAYASHI**[1,2], N. L. HOHERCHAK[1], R. B. IVRY[1];
[1]Psychology, Univ. of California, Berkeley, Berkeley, CA; [2]Grad. Sch. of Frontier Biosci., Osaka Univ., Suita, Japan

Abstract: The ability to precisely estimate time intervals is crucial in many aspects of perception and action. A recent neuroimaging study suggested that time perception may be mediated by a neural population tuned for specific durations: Regions in the right parietal cortex showed duration-specific repetition suppression (Hayashi et al., 2015 *PLoS Biology*). This hypothesis is further supported by psychophysical work showing that adaptation to a specific duration produces a negative aftereffect: Stimulus durations are over-estimated following exposure to a short adaptor and under-estimated following exposure to a long adaptor. Here, we show that adaptation to a specific duration produces, not only systematic biases in perceived durations for longer and shorter durations than the adaptor, but also improvements of sensitivity for the adapted duration. Participants were tested on two perceptual discrimination tasks. On each trial, they were presented with an auditory white noise of 500 ms (standard duration) and asked to judge if a visual stimulus of variable duration (comparison duration, 200 - 800 ms) was shorter or longer than the standard (Experiment 1) or same or different (Experiment 2). In adaptation blocks, the perceptual judgments were preceded by an adaptation period in which a visual stimulus of 500 ms duration was repeatedly presented. In baseline blocks, the adaptation period was absent. We used a cross-modal comparison (auditory standard/visual comparison) so that the effects of adaptation would be limited to the comparison stimulus. In both experiments, there was no change in the point of subjective equality. However, adaptation resulted in a steeper psychophysical function compared to the no-adaptation condition. Moreover, in the same-different judgment task (Experiment 2), adaptation condition showed a higher accuracy in judging the two stimuli as “same” when both stimuli were of 500 ms duration, suggesting that repeated exposure to a specific duration produces higher sensitivity for that duration. The

duration-specific repetition suppression found in the previous neuroimaging study may reflect sharpening tuning curves of duration tuned units rather than neural fatigue/saturation.

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Poster

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Topic: H.02. Human Cognition and Behavior

Title: A cerebellar role in optimal sub-second trajectory prediction

Authors: ***C. B. OWENS**[1,2], C. DE BOER[1], G. GENNARI[3,1], R. BROERSEN[4], J. PEL[1], J. VAN DER STEEN[1,5], W. CLAPP[5], B. MILLER[5], Y. VAN DER WERF[6], C. DE ZEEUW[1,4]; [1]Neurosci., Erasmus MC, Rotterdam, Netherlands; [2]Agency for Science, Technol. and Res., Singapore, Singapore; [3]Neurosci., Univ. of Padua, Padua, Italy; [4]Netherlands Inst. for Neurosci., Amsterdam, Netherlands; [5]Neuroscouting LLC, Cambridge, MA; [6]VU Univ. medical center, Amsterdam, Netherlands

Abstract: Professional athletes like soccer, tennis or baseball players possess superior perceptual, predictive and decision-making skills. However, it is unclear what neural systems play a role and to what extent they utilize both internally (endogenous) and externally (exogenous) generated temporal cues to optimize trajectory prediction of the ball. We subjected expert baseball players and controls to task conditions faced while at bat. While observing a stimulus moving vertically across a touchscreen towards a visible square target, subjects had to predict whether the ball would terminate inside the target prior to the end of its trajectory. Endogenous (fixation time) and exogenous (stimulus movement, speed, and expansion) cues were varied across all trials. Experts were more successful than controls when temporal cue conditions were most challenging. When preparatory time was short, experts allocated attentional resources more quickly, as indicated by faster pupillary responses. When stimulus speed was fast experts exhibited optimal voluntary saccadic eye movements at the early stage of the task. Under both conditions experts exhibited superior performance. Using an fMRI version of the task we found good performance was associated with a brain network consisting of sensorimotor cortex, inferior parietal lobe and cerebellum. These results indicate that 1) experts rely more heavily on endogenous alerting temporal cues than controls, 2) experts utilize externally generated cues more efficiently than controls and 3) the cerebellum is critical to optimal sub-second trajectory prediction.

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Poster

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Title: The effects of age & interaural delay on human cortical processing of changes in interaural correlation

Authors: ***M. WANG**, Y. YANG;
Sch. of Psychology, Beijing Normal Univ., Beijing, China

Abstract: When the listening situation is complex (e.g., when there are many people talking at the same time), older adults often complained that it's difficult to process and comprehend what a target talker is saying, even with the normal hearing. Several studies have indicated the subjective representation of the sounds delivered to the two ears of a human listener is closely associated with the interaural correlation (IAC) and interaural delay of these two-ear sounds. The age-related changes in hearing may make it more difficult for older adults to analysis IAC. This study compares the cortical processing ability of younger and older adults to processing the changes in interaural correlation, by using event-related potential technology. Here we show that participants in older group behaviorally responded slower to a change in IAC than those in younger group only at large interaural delay (6 ms). However, the response speed of older group was the same as those of younger group when the interaural delay is smaller (0 ms, 2 ms and 4 ms). We then recorded the cortical response to the interaural correlation change using event-related potentials. On the contrary, our cortical processing records showed that participates in the older group had longer P2 latency than those in the younger adults group, even with the smaller interaural delay. Accordingly, P2 amplitude of the older group was also smaller than that of the younger group. In addition, participates in the older group had larger N1 amplitude than those in the younger group. The difference between two age groups increased in proportion to the increasing of interaural delay. These results suggest that human's cortical processing of changes in IAC is affected by aging. This age-related deficit appears to be expected as temporal jitter

(loss of neural synchrony in the auditory system) increases with interaural delay. The mismatching between the behavior and cortical results implicated that the perceptual cues could help older adults to overcome this deficit to a certain extent, while the test of cortical processing could reveal the deficit much earlier and clearer.

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Poster

747. Plasticity and Disorders of Executive Function

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Support: Intelligence Advanced Research Projects Activity (IARPA) Grant 2014-13121700007

Title: FAST: a novel, executive function-based approach to cognitive enhancement

Authors: *J. N.-F. ALMQUIST[1], S. MATHAN[1], A.-K. BREM[2], F. PLESSOW[3], J. MCKANNA[4], A. PASCUAL-LEONE[3], R. COHEN KADOSH[2], M. PAVEL[4], D. CORNHILL[1], M. DILLARD[1], D. ERDOGMUS[4], G. KIMBALL[5], K. MANSFIELD[2], E. MYERS[5], U. ORHAN[1], E. SANTARNECCHI[3], T. THOMPSON[3], N. YEUNG[2];
[1]Honeywell Labs, Honeywell Aerospace, Redmond, WA; [2]Dept. of Exptl. Psychology, Univ. of Oxford, Oxford, United Kingdom; [3]Berenson-Allen Ctr. for Non-Invasive Brain Stimulation & Div. for Cog Neurology, Dept. of Neurol., Beth Israel Deaconess Med. Center, Harvard Med. Sch., Boston, MA; [4]Electrical and Computer Engin. Dept., Northeastern Univ., Boston, MA; [5]SimCoach Games, Pittsburgh, PA

Abstract: The present study introduces a novel cognitive intervention aimed at improving fluid intelligence (G*f*), based on what we call the FAST framework: Flexible, Adaptive, Synergistic Training. FAST leverages a combination of novel executive function-based training and transcranial electrical stimulation, with aims to synergistically activate and strengthen mechanisms of cognitive control critical to G*f*. The question of whether targeted training of cognitive skills can enhance G*f* is a topic of intense debate, and is one of substantial practical and theoretical importance. This study aimed to assess whether the FAST intervention could lead to enhancement of G*f*, relative to both a no-contact and an active control condition. To test our intervention we collected three G*f* measures from 113 participants (BOMAT, Raven's Advanced Progressive Matrices, and matrices similar to those in Raven's, generated by Sandia labs), prior to and following one of three interventions: (1) the FAST intervention, a combination of 30 minutes of daily training with our novel training game, Robot Factory, and 20 minutes of

concurrent transcranial random noise stimulation applied to bilateral dorsolateral prefrontal cortex, (2) an adaptively difficult active control intervention comprised of visuospatial tasks that specifically do not target G*f*, or (3) a no-contact control condition. Logistic regression analyses of performance at posttest, controlling for pretest performance and age, found that the FAST group performed significantly (vs. No-contact) or marginally better (vs. Active Control) in terms of number of items answered correctly across all three G*f* posttests, and significantly better in terms of accuracy (number of correct responses/number of items attempted) against both controls. This enhanced performance was found to be driven by significantly better performance for FAST in the BOMAT (and to a lesser extent Raven's), empirically the most difficult of our tests, indicating performance improvements from potential transfer may be most apparent on more difficult G*f* test items. A final analysis of the FAST group alone found a significant correlation between progress in Robot Factory and posttest performance, implicating the role of FAST training in G*f* enhancement.

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Poster

747. Plasticity and Disorders of Executive Function

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 747.02/KKK50

Topic: H.02. Human Cognition and Behavior

Title: Alterations in resting state connectivity within the central executive network in HIV predict neurocognitive impairment

Authors: *R. C. MCINTOSH[1], M. HIDALGO[2], C. SHIKUMA[3], K. KILLIANPUR[4]; [2]Psychology, [1]Univ. of Miami, Coral Gables, FL; [4]Med., [3]John A. Burns Sch. of Med., Honolulu, HI

Abstract: Research suggests that HIV impacts the intrinsic connectivity of functional brain networks at rest. HIV-related deficits have also been identified within a cortico-striatal-thalamo-cortical loop that is recruited during rule-based learning, sequence learning, working memory and planning. This investigation sought to evaluate whether differences in resting-state functional connectivity between 33 HIV-positive (mean age 53.2 years) and 36 HIV-negative

adults (mean age 56.8 years) predicts global neurocognitive function using functional networks derived from independent component analysis (ICA). Resting-state fMRI data was concatenated in time across subjects to create a single 4D dataset and decomposed into 30 ICA maps using Multivariate Exploratory Linear Optimized Decomposition. ICA components were then reconstructed for each subject's 4D data to estimate subject-specific spatial maps using the dual-regression technique. A total of 15 components were identified as noise and removed. The other 15 ICA components were matched with previously established networks. Comparisons between HIV+ and HIV- controls revealed significant contrasts at the 0.01 level. Of the 15 identified components between group differences were observed in the left parietal attention network, left ventral attention network, frontal cortical network, sensory-motor network and central executive network (CEN). To evaluate whether the functional integrity of the CEN relates to global neuropsychological function time courses from seeds within the left and right prefrontal cortex, caudate body, and medial dorsal nucleus of the thalamus (MDT) was correlated with individual subject-level z-scores. Although left and right prefrontal cortex and caudate body did not correlate with global neuropsychological function, connectivity of the right MDT with the CEN was negatively associated with neurocognitive function in HIV+ individuals ($\beta = -0.43$, $p = 0.02$) while the left MDT showed a positive association for the control group ($\beta = 0.363$, $p = 0.04$). After controlling for age, CD4 count and undetectable viral load status right MDT maintained a negative correlation with global neuropsychological function in the HIV+ group ($\beta = -0.60$, $p = 0.001$). This pattern suggests lateralization of MDT recruitment within network designated for cognitive task performance. Furthermore, the absence in left MDT recruitment, as it corresponds to global neuropsychological function, suggests that HIV alters functional connectivity of the CEN. Resting-state connectivity may be a useful biomarker for disorganization of networks that contribute to HIV-associated neurocognitive dysfunction.

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Poster

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Topic: H.02. Human Cognition and Behavior

Support: the U.S. Army Medical Research and Material Command under Contract No. W81XWH-14-C-0018

Title: Second validation of a system to monitor, extract, and decode indicators of cognitive workload (MEDIC)

Authors: *B. K. BRACKEN[1], A. NEGRI[2], P. AMAZEEN[3], M. FEDELE[4], A. LIKENS[3], M. DEMIR[4], N. PALMON[2], S. ELKIN-FRANKSTON[2], N. COOKE[4];
[1]Cognitive Systems Div., [2]Charles River Analytics, Cambridge, MA; [3]Arizona State Univ., Tempe, AZ; [4]Arizona State Univ., Mesa, AZ

Abstract: Emergency medical personnel must act quickly and effectively, both as individuals and as teams. Medical training often includes high-fidelity simulations. However, trainers infer competence by observation alone—a challenging task. We designed and are validating our system to augment training by Monitoring, Extracting, and Decoding Indicators of Cognitive Workload (MEDIC). MEDIC includes: (1) a suite of unobtrusive, field-ready neurophysiological, physiological, and behavioral sensors, including a user interface (UI) for trainers to enter notes during the scenario on team metrics and when important events occur; (2) a Data Processing and Fusion Engine to process and time-align raw data originating from the sensor suite; (3) a Data Interpretation Engine to interpret the best indicators of cognitive workload and team dynamics; and (4) an after-action review UI to display interpreted human states time-aligned with pertinent events that occurred across the scenario. During this validation study, teams of three Arizona State University participants completed an obstacle course of physical and cognitive challenges including (1) memorizing word lists, (2) balancing moving balls on weighted boards, (3) assembling puzzles, (4) passing weighted medicine balls while balancing on Bosu balls, (5) completing logic problems, (6) constructing walls using boxes of different sizes and weights, and (7) jumping rope in sync with other team members. Our data processing and fusion engine de-noises, processes, and time-aligns data from all sensors, then pushes data through our data interpretation engine that uses statistical and probabilistic modeling techniques to output estimates of individual state (e.g., stress and cognitive workload) and team state (e.g., team dynamics). Our next validation is at a simulation center at Vanderbilt Medical School where we will collect data on medical residents during high-fidelity simulations. This work was supported by the U.S. Army Medical Research and Material Command under Contract No. W81XWH-14-C-0018. The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

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Poster

747. Plasticity and Disorders of Executive Function

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Program#/Poster#: 747.04/KKK52

Topic: H.02. Human Cognition and Behavior

Title: ERP as a biomarker for tDCS neuromodulation of executive function

Authors: *L. DUBREUIL VALL[1,2,3], P. CHAU[2], G. RUFFINI[1], J. CAMPRODON[2]; [1]Neuroelectrics, Cambridge, MA; [2]Massachusetts Gen. Hosp., Charlestown, MA; [3]Univ. de Barcelona, Barcelona, Spain

Abstract: Background
Executive function is an umbrella term encompassing subdomanins such as attention, working memory and inhibitory control. Recent studies suggest that transcranial direct current stimulation (tDCS) targeting the DLPFC modulates these functions, which are disrupted across the neuropsychiatric spectrum. In the present study, we investigated the role of tDCS in modulating attention and working memory in healthy adults.
Methods
In this crossover study, 20 subjects received 3 different tDCS stimulation conditions over 3 separate visits: Sham, anodal tDCS on the right DLPFC (Anodal Right), and anodal tDCS on the left DLPFC (Anodal Left) using 3cm^2 Ag/AgCl electrodes. The return electrode (cathode) was placed in the contralateral supraorbital region. Participants performed the Flanker and MSIT-IAPS tasks before and after receiving tDCS. In addition to measuring behavioral responses, we recorded EEG and calculated the ERPs over Fz for all tasks.
Results
For the **Flanker task**, we found that Anodal Left stimulation led to a significant increase of P300 amplitudes for incongruent trials versus Sham, which led to significant amplitude decrease. P300 amplitude is associated with attention resource allocation during working memory updating; larger P300 amplitudes indicate greater attention allocation. This is consistent with the behavioral results, which show Anodal Left stimulation led to a significant improvement in reaction time (RT) and a nearly significant improvement in accuracy versus Sham, which led to a smaller increase in RT and a significant decrease in accuracy.
In the **MSIT-IAPS** task**,** the strongest finding is that Anodal Right and Left stimulation significantly increased P600 amplitude versus Sham, which did not have a significant effect. P600 is an index of synchronized operation completion after target detection, having much in common with working memory operation. This is consistent with the behavioral performance, in which Anodal Left stimulation led to a greater improvement in accuracy and RT versus Sham, which led to a smaller improvement.
Conclusions

Our results show that specific working memory and attention-related ERPs are modulated by anodal tDCS applied over DLPFC in healthy adults. This modulation is correlated with an improvement in the behavioral performance, suggesting tDCS as a possible method to improve executive function. Furthermore, the correlation between behavioral performance and ERP modulation presents these ERPs as potential biomarkers and therapeutic targets of executive function modulation.

Disclosures: **L. Dubreuil Vall:** A. Employment/Salary (full or part-time): Neuroelectrics. **P. Chau:** None. **G. Ruffini:** A. Employment/Salary (full or part-time): Neuroelectrics. **J. Camprodon:** None.

Poster

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Program#/Poster#: 747.05/KKK53

Topic: H.02. Human Cognition and Behavior

Title: Selective attention in women victims of domestic violence

Authors: B. BEDOLLA[1], J. NUÑEZ[1], *M. M. LOPEZ-TITLA[2], D. ANGELES[3]; [1]Univ. del Valle de México, MEXICO, Mexico; [2]IMAGENES CEREBRALES, INPRFM, MEXICO, Mexico; [3]Facultad de psicología. UNAM, MEXICO, Mexico

Abstract: Domestic violence has increased in Mexico in the last years. Statistics shows that 47 percent of women (15 years old and older) have experienced some kind of violence by their partner [1]. Previous studies have found that this types of stressful events could cause an alteration in women cognitive process involving executive functions. Muriel Lezak defined these functions as the essential mental abilities to carry out an effective, creative and socially accepted behavior [4,5]. And Mateer suggests that executive functions consist on: direction of attention, pattern recognition priority, formulation of intention, plan achievement or accomplishment and implementing the plan and recognition of achievement [6]. Selective attention and cognitive flexibility are associated with the processing of emotional cues in the ventromedial circuit. The former process guide our decision making towards objectives based on social and ethical judgment[3]. To asses the selective attention performance on women victims of domestic violence we designed two Go/no Go[2] task variants in the software E-prime (version 1.1). Both tasks show several stimulus and the subject must respond to only one kind of stimulus. To discard the size and position stimulus effect, the stimulus in the first task were placed in the center of the screen and all have the same size. In the second task the stimulus changes its position on the screen and its size differs. These tasks were validated by healthy subjects without

psychiatric or psychologic history. The subjects were evaluated with the mini mental test, SCL-90R scale, Rosenberg Self Esteem Scale and the Beck Depression Scale score. The error rate for the first task is 5.10% and for the second task is 8.74% then the error rate for the first task is less than for the second. For that reason we stipulate that the position and the size of the stimulus do matter in the performance of the selective attention of the subjects.
1. Panorama de violencia contra las mujeres en México: ENDIREH 2011 / Instituto Nacional de Estadística y Geografía.-- México: INEGI, 2013. 2.Elliot Kale Edmiston and Jennifer Urbano Blackford. Childhood maltreatment and brain response to novel faces in adults with inhibited temperament.HHS Public Access. 20033.Lezak MD. Relationship between personality disorders, social disturbances and psysical disability following traumatic brain injury. J Head Trauma Rehabil; 1987. 4. Lezak MD. The problem of assessing executive functions. Int J Psychol; 1982. 5. Junqué C, Barroso J. Neuropsicología. Madrid: Síntesis; 1994. 6. Sholberg MM, Mateer CA. Remediation of executive functions impairments New York: Guilford Press; 1989.

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Poster

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Topic: H.02. Human Cognition and Behavior

Support: K99-R00 MH096801

Title: Cognitive control network flexible hub connectivity is altered across distinct mental illnesses

Authors: ***M. SPRONK**[1], A. ANTICEVIC[2], M. W. COLE[1];
[1]Rutgers Univ., Newark, NJ; [2]Yale Univ., New Haven, CT

Abstract: It has recently been suggested that flexible hubs in the human brain's cognitive control networks (CCNs) regulate other brain networks in a goal-directed manner for optimal behavioral outcomes in healthy populations, thereby promoting mental health. In line with this, behavioral and neuroimaging studies have found impaired cognitive control abilities in various mental illnesses, but the underlying neural mechanisms remain unclear. One possibility is that CCN dysfunction is related to altered brain-wide connections of flexible hub regions in CCNs (i.e. in cingulo-opercular, frontoparietal and dorsal attention networks), which could affect CCN capacity in various mental illnesses, eventually leading to impaired regulation of symptoms in clinical compared to healthy individuals. A still outstanding question is whether there are

common alterations in connectivity of CCNs regions across different mental illnesses. In this study, we therefore try to clarify the nature of cognitive control deficits in a range of mental illnesses and across the lifespan. To test the hypothesis that CCN dysfunction may be a common factor in a broad range of mental illnesses and that CCN flexible hubs exhibit comparable altered connections across various clinical populations, we used resting state functional connectivity and graph theoretical measures. More specifically, resting state fMRI data from various publicly available sources including data from subjects with different mental illnesses and age groups (such as ADHD, autism and pre-Alzheimer's disease) were analyzed, and hub-characterizing measures such as global brain connectivity and betweenness centrality were obtained to characterize network architecture of CCN regions. Using these measures we indeed found altered brain-wide connectivity of CCN regions in clinical compared to healthy control groups. Furthermore, we achieved above-chance classification of clinical subjects versus healthy controls based on resting state functional connectivity of the CCNs. These results shed new light on commonalities in the neural mechanisms of cognitive control dysfunction across a broad range of mental illnesses. Finding common factors in control system dysfunction could improve our understanding of the underlying mechanisms of mental illness and have important implications for therapeutic interventions.

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Center for Nutrition Learning and Memory, UIUC (2012-04673) to AFK & EM

Title: Brain network predictors of training-related gains in older adults after exercise intervention

Authors: *P. L. BANIQUED[1], C. L. GALLEN[1], M. W. VOSS[2], C. N. WONG[3], G. E. COOKE[3], A. Z. BURZYNSKA[4], K. DUFFY[3], J. FANNING[3], D. EHLERS[3], E. AWICK[3], E. MCAULEY[3], A. F. KRAMER[3], M. D'ESPOSITO[1];
[1]Helen Wills Neurosci. Inst., Univ. of California Berkeley, Berkeley, CA; [2]Univ. of Iowa, Iowa City, IA; [3]Univ. of Illinois at Urbana-Champaign, Urbana, IL; [4]Colorado State Univ., Fort Collins, CO

Abstract: Recent work suggests that the brain can be conceptualized as a network comprised of groups of sub-networks or modules. The extent of modules' segregation can be quantified with a modularity metric, where networks with high modularity have dense connections within modules and sparser connections between modules. Previous work has shown that higher baseline modularity predicts greater improvements after cognitive training in patients with traumatic brain injury, and more recently, in healthy older and young adults. It is not known however, whether modularity can similarly predict cognitive outcomes after a physical exercise intervention. Here, we quantified modularity in a subset of older adults (n=127) who underwent one of the following interventions for 6 months: 1) aerobic exercise (AERO), 2) aerobic exercise plus nutritional supplement (AERO+), 3) stretching, strengthening and stability (SSS), or 4) dance instruction. After the intervention, the AERO, AERO+ and SSS groups showed gains in cardiorespiratory fitness, with larger effects in both aerobic groups compared to the SSS and dance groups. The AERO, AERO+ and SSS groups also improved in cognitive control as measured by reasoning, working memory, and task-switching tests. We derived brain networks using fMRI data from a 6-minute resting state scan collected prior to training, and computed modularity from functional connectivity matrices generated using 264 regions of interest. In the aerobic and SSS subjects that improved in physical fitness and cognitive control, higher baseline modularity was positively related to cognitive control gains, even after controlling for age, years of education, and in-scanner motion. No relationship between modularity and cognitive control gains was observed in the dance group, which did not show training-related gains in cognitive control. Since previous studies have found that the relationship between modularity and training gain is driven by lower performing individuals, we performed a median split in the aerobic and SSS groups based on baseline fluid reasoning ability. The positive relationship between modularity and cognitive control gains was evident only in the groups with lower baseline reasoning ability. These results are in line with previous studies that find that individuals with a more modular brain structure are more responsive to cognitive training. More generally, these findings suggest that the predictive power of modularity may be generalizable across interventions aimed to enhance aspects of cognition and that, especially in low-performing individuals, global network properties can capture individual differences in neuroplasticity.

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Poster

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Topic: H.02. Human Cognition and Behavior

Support: NIH Grant AA016624

SDSU

Title: Binge drinking and neural oscillatory dynamics underlying response inhibition

Authors: *L. A. HOLCOMB[1], S. HUANG[1], S. M. CRUZ[1], L. C. WAGNER[1], A. ANDREWS[1], K. MARINKOVIC[1,2];
[1]Psychology, San Diego State Univ., San Diego, CA; [2]Radiology, UCSD, San Diego, CA

Abstract: Alcohol consumption in young adults is often characterized by heavy episodic, or binge drinking, which is commonly defined as heavy consumption on a single occasion and is associated with a multitude of health risks and social problems. Response inhibition refers to the ability to withhold or suppress prepotent actions and is an important component of executive functions. Previous evidence indicates that response inhibition is affected by acute intoxication as well as by long-term alcohol abuse. Furthermore, it is highly pertinent to binge drinking as impaired response inhibition predicts engaging in binge drinking and is a risk factor for developing alcohol and substance abuse. The current study was designed to investigate the neural dynamics of response inhibition as a function of binge drinking in young adults. Participants were 45 (25 women) healthy, right-handed subjects with a mean age (± st. dev) of 23.64 ± 3.57 years who were prescreened for alcohol use to determine eligibility. Binge and Control participants differed greatly in a number of drinking patterns. Binge subjects reported more binge episodes (defined as consuming 6+ for men or 5+ for women drinks within two hours), blackouts, more drinks consumed per drinking occasion, and drank on more days throughout the week than Controls. Binge subjects also reported higher anxiety, impulsivity, and depression than Control subjects. During a Go/Nogo task, participants were instructed to respond to a prepotent stimulus (80% of the time), and to occasionally withhold response (20% of the time). Scalp EEG was recorded with a 64-channel BrainVision system, and a Morlet wavelet transform for theta (4-7 Hz) frequency was applied. Results suggest that Binge participants responded impulsively to the task, as they committed marginally more premature responses (ones made too early) than Control participants. Indeed, self-reported impulsivity and drinking levels correlated positively with the inability to withhold responses in Binge participants. Theta oscillations peaked at ~350 ms after stimulus onset and were greater to NoGo trials overall. Event-related theta power was marginally reduced in the Binge participants at central and parietal scalp locations from 300-400 ms in NoGo trials. However, no group differences were seen in Go trials.

This suggests that a history of binge drinking is associated with impaired response inhibition as reflected in theta oscillations, which may further contribute to the inability to refrain from harmful levels of drinking. These results are consistent with and extend prior reports of decreased theta in chronic alcoholics and those under acute intoxication.

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Poster

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Topic: H.02. Human Cognition and Behavior

Support: NIH AA016624

SDSU

Title: Neurodynamics of decision making under conditions of high and low response conflict as a function of binge drinking

Authors: *S. CRUZ[1], S. HUANG[1], L. A. HOLCOMB[1], L. E. BEATON[1], N. M. FONG[1], K. MARINKOVIC[1,2];
[1]Psychology, San Diego State Univ., San Diego, CA; [2]Radiology, UCSD, San Diego, CA

Abstract: The ability to override automatic responses with new, adaptive behavior when presented with conflict is an essential function of cognitive control. Previous research has shown that cognitive control is diminished by long-term excessive drinking and during acute alcohol intoxication especially within the prefrontal network. However, despite great prevalence of high risk alcohol consumption in young adults, studies investigating the persistent neurophysiological effects of heavy alcohol consumption are still scarce. The purpose of this study was to investigate brain indices of cognitive control during a modified Stroop naming task in young adults as a function of heavy episodic (binge) drinking pattern. Forty-five (25 women) healthy, right-handed subjects (23.6 ± 3.5 yrs) were screened for their alcohol consumption habits. Twenty-two subjects reported heavy drinking patterns with 11.5 ± 8.4 binge episodes during the last six months, compared to control subjects who had 0.1 ± 0.3 binge episodes. In addition, binge participants reported more blackouts, higher levels of alcohol consumption, higher rates of anxiety, impulsivity and depressive symptoms than control participants. Subjects performed a modified Stroop naming task which elicited high and low levels of response conflict on

congruent and incongruent trials respectively. Continuous EEG signal was recorded with a 64-channel Brain Vision acquisition system and analyzed in both time- and time-frequency domains. The Stroop interference effect was reflected in lower accuracy and slower response times on incongruent trials overall. Binge participants tended to respond more impulsively as shown by higher levels of self-corrected responses. Event-related potentials (ERPs) analysis revealed a smaller N250 deflection in the binge group across all task conditions. The subsequent late positivity tended to be greater in the binge drinkers only on high conflict trials frontocentrally. Event-related theta power (4-7 Hz) was calculated with Morlet wavelets. Incongruent trials evoked the greatest theta power overall confirming that it is a good index of increased demands on cognitive control. Compared to controls, binge drinkers tended to have higher theta power on congruent trials over the frontal sites, possibly suggesting impaired flexibility in cognitive control of resource allocation in situations evoking response conflict. Taken together, these results indicate that binge drinking is associated with impairments of the prefrontal network that subserves decision making under conflict conditions. This impairment could contribute to deficits in self-control and increased drinking.

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Poster

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Topic: H.02. Human Cognition and Behavior

Support: CONACyT 221092

CONACyT 238313

Title: Cortisol levels and inhibitory control correlated with relapse on a group of addicts in rehab treatment

Authors: ***Y. BENITEZ**[1], Y. RUVALCABA-DELGADILLO[2,3], T. MORALES-SALCEDO[2], A. AGUILAR-DELGADILLO[2], A. LARIOS-RUIZ[2], T. VILLASEÑOR-CABRERA[1], F. JAÚREGUI-HUERTA[2];
[1]Programa de Maestría en Neuropsicología, Univ. De Guadalajara, Guadalajara, Mexico; [2]Dept. de Neurociencias, Univ. de Guadalajara, Guadalajara, Mexico; [3]Ctr. de Tratamiento Volver a Nacer A.C., Guadalajara, Mexico

Abstract: Addiction is a chronic disease characterized by the compulsive use of drugs. The treatments are not very effective; in fact, 60%-70% of the addicts relapse in the first year of abstinence. Stress and inhibitory control are a risk factor in both consumption and in relapse. In this investigation a group of addicts in rehab were exposed to a model of experimental stress, where the cortisol levels and the inhibitory control ability were assessed in order to correlate them with the incidence of relapse in drug consumption, once rehab treatment was concluded. The Trier Social Stress Test (TSST) was used to produce moderate stress in 60 subjects almost concluding the rehab treatment. Cortisol levels were measured before, during and after TSST, by enzyme immunoassay. In addition, interference control (an ability of the executive function inhibitory control), was evaluated with the Stroop test before and during the TSST. Once the subjects finished the rehab treatment, they were monitored during 1 year in order to register the relapse incidence. 81% of the sample was male and 19% women. The average age for those subjects was 24.6 (SD=5.022) with 11.6 (SD=3.047) years of education. The most used drugs were methamphetamines (51.5%), followed by alcohol (21.2%), cocaine (15.2%), marijuana (9.1%), and inhalants (3%). The mean term of consumption was 4.15 (SD= 3.27). Our preliminary results indicate that in one-year follow-up, incidence of relapse is up to 50%. Evaluated subjects also show differences in interference control before and after the stress stimulus. Changes in cortisol patterns were also observed through time in these subjects. Then, we believe that cortisol fluctuations and interference control may be useful tools trying to predict relapse.

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Poster

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Topic: H.02. Human Cognition and Behavior

Title: Validity of an online neuropsychological assessment, the NCPT

Authors: *E. CORDELL, N. NG, K. KERLAN, C. SIMONE, G. MORRISON;
Res., Lumos Labs, San Francisco, CA

Abstract: The ability to measure neuropsychological functioning across the lifespan is critical for advancing our understanding of normal, healthy aging and age-related cognitive disorders such as MCI, dementia, and Alzheimer's. The ability to measure an individual's

neuropsychological performance in an actionable and informative way remains an imperative for research studies, clinical diagnoses, and intervention monitoring. However, present day approaches for measuring cognitive performance are lengthy, expensive, and burdensome. The NeuroCognitive Performance Test (NCPT; Lumos Labs, Inc.) is a brief online battery of cognitive assessments used to measure functioning in five cognitive domains. The NCPT is being developed for use in clinical research - as an outcome measure and for screening participants for entry into research trials - and for use in a clinical setting to aid in the diagnosis of cognitive impairment and longitudinal monitoring of cognitive change over time. As with the development of any new assessment or intervention, it is imperative that novel assessments first be validated against conventional present-day evidence-based best-practices. The goal of this study was to evaluate the validity and reliability of the NeuroCognitive Performance Test (NCPT) compared to corresponding conventional pencil-and-paper neuropsychological assessments. This was a multi-site, randomized, counterbalanced study that enrolled 234 healthy adults between the ages of 20-79 (inclusive). The NCPT and conventional neuropsych assessments were conducted at both study visits. In this poster, we will report the NCPT subtests that were used, and their corresponding pencil-paper neuropsychological assessments. Our data analysis will explore the correlations between (1) the NCPT subtests and their pencil-paper correlates and (2) the NCPT total score (known as the Grand Index) and the neuropsych composite scores. Secondly, we will also report test-retest reliability of the NCPT and neuropsych assessments. We anticipate the ability to also report exploratory analyses displaying any significant age, gender, or education-level group differences.

Disclosures: **E. Cordell:** A. Employment/Salary (full or part-time): Lumos Labs. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Lumos Labs. **N. Ng:** A. Employment/Salary (full or part-time): Lumos Labs. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Lumos Labs. **K. Kerlan:** A. Employment/Salary (full or part-time): Lumos Labs. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Lumos Labs. **C. Simone:** A. Employment/Salary (full or part-time): Lumos Labs. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Lumos Labs. **G. Morrison:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Lumos Labs. F. Consulting Fees (e.g., advisory boards); Lumos Labs.

Poster

747. Plasticity and Disorders of Executive Function

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 747.12/KKK60

Topic: H.02. Human Cognition and Behavior

Support: Swiss National Science Foundation 320030_159705

Title: The cortical-subcortical circuit dynamics of OCD and Tourette syndrome as revealed by functional connectivity analysis of simultaneous scalp and depth EEG recordings

Authors: *R. HASHEMIYOON[1], N. PRINS[2], A. COITO[2], M. TOMESCU[2], T. SCHÜLLER[1], T. SCHÜLLER[1], E. SILDATKE[1], J. KUHN[1], V. VISSER-VANDEWALLE[1], C. MICHEL[2]; [1]Univ. Hosp. of Cologne, Cologne, Germany; [2]Univ. of Geneva, Geneva, Switzerland

Abstract: Neuropsychiatric disorders are prevalent in society. Despite the pervasiveness of these illnesses, our understanding of the biological basis of these disorders is severely limited. Two such disorders are Gilles de la Tourette syndrome (GTS) and obsessive compulsive disorder (OCD). Neither of these is well understood in terms of their etiology nor pathomechanisms; however, dysfunction in the circuits of the cortico-basal ganglia-thalamo-cortical network is a suggested source of their respective pathologies. This network regulates motor, emotional, and cognitive function. Perturbations in the balance of its functional connections arguably manifest as the cognitive, motor, and/or limbic deficits seen in GTS and OCD. It is suggested that similar circuit dysfunction underlies these disorders, accounting for their high comorbidity rate; yet, their profiles are asymmetric. In addition to ~ 100% difference in population frequency, GTS occurs with a near 80% comorbidity rate with OCD, while OCD is reported to occur at about 7% comorbidity with GTS. These variances could be pivotal to understanding the foundational mechanisms governing the two disorders. In order to explore the interplay between the subcortical nuclei and the cortex in the large scale network, we recorded from a cohort of male and female OCD and GTS patients (aged 27-55 years old) who had undergone surgery for deep brain stimulation (DBS). Electrophysiological recordings were taken simultaneously from multi-channel scalp EEG combined with externalized DBS electrodes (allowing invasive access to deep structures) bilaterally implanted in the nucleus accumbens or centromedial nucleus of the thalamus, respectively. Effective functional connectivity measures were calculated during the resting state using partial directed coherence based on Granger causality. In all patients, local circumscribed networks surrounding specific subcortical electrodes were identified as major drivers of the large-scale subcortical-cortical networks. They projected to specific electrode sites on the scalp. In turn, some scalp electrodes strongly communicated with the subcortical structures. Interestingly, while the dominant frequency in all electrodes was in the high theta/low alpha range, the subcortical and cortical electrodes had different and preferred frequencies of

communication. Our cortical-subcortical simultaneous recordings provide the platform for a deeper understanding of the temporal dynamics of whole-brain networks in humans, which includes the intimate contribution of deep structures and opens new ways of identifying neurophysiological markers of these psychiatric diseases.

Disclosures: **R. Hashemiyoon:** None. **N. Prins:** None. **A. Coito:** None. **M. Tomescu:** None. **T. Schüller:** None. **T. Schüller:** None. **E. Sildatke:** None. **J. Kuhn:** None. **V. Visser-vandewalle:** None. **C. Michel:** None.

Poster

747. Plasticity and Disorders of Executive Function

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Topic: H.02. Human Cognition and Behavior

Support: Grant-in-Aid for JSPS Fellows

Title: Exercise-induced cognitive fatigue and its brain mechanism in normobaric hypoxia: A neuroimaging study

Authors: ***G. OCHI**[1,2], K. HYODO[1,3], K. SUWABE[1], H. SOYA[1];
[1]Lab. of Exerc Biochem & Neurosci, Univ. of Tsukuba, Tsukuba-Shi, Japan; [2]Res. Fellow of Japan Society for the Promotion of Sci., Tokyo, Japan; [3]Physical Fitness Res. Institute, Meiji Yasuda Life Fndn. of Hlth. and Welfare, Hachioji-City, Japan

Abstract: Exercise-induced fatigue consists of central fatigue, with symptoms such as a reduction of central motor command, and the peripheral fatigue, which occurs in muscles. Exercise-induced central fatigue may also lead to deteriorating cognitive function (cognitive fatigue) mediated by the prefrontal cortex, which carries out executive function, and diminished athletic performance. Although the neural mechanisms behind cognitive fatigue associated with central fatigue are still unclear, cerebral hypoxia is thought to be an important factor. We thus aimed to create an experimental model for exercise-induced cognitive fatigue using hypoxic exercise and to identify the underlying neural mechanisms using multichannel functional near-infrared spectroscopy (fNIRS). Sixteen healthy young adults (mean age 20. 7 ± 1.9 years) participated in this study. They performed a color-word Stroop task (CWST) before and 15 minutes after 10 minutes of moderate exercise (50% VO_2peak; EX) or rest (CON) under moderate hypoxic conditions (13% O_2). Cognitive performance was assessed by reaction time (RT) for the CWST. Difference in RT between incongruent and neutral trials were calculated as $RT_{interference}$ to determine executive function. fNIRS probes were put over the forehead during the

CWST and we monitored brain activity on both sides of the dorsolateral prefrontal cortex (DLPFC), which plays a crucial role in executive function. Task-related oxy-Hb concentration changes were used as indicators of brain activity. Arterial oxygen saturation (SpO_2) was monitored throughout. Results showed that SpO_2 decreased with hypoxia in EX and CON (EX: 87.9±3.3%, CON: 89.9±2.7%, N.S.). In addition, SpO_2 decreased during exercise (81.9±2.1%) and recovered to pre-exercise level after exercise. A two-way ANOVA showed significant interactions between time (pre/post) and condition (EX/CON). Post-hoc analysis revealed that exercise delayed $RT_{interference}$ and decreased task-related oxy-Hb concentration changes in the left DLPFC compared to CON. The Spearman correlation test revealed a negative correlation between $RT_{interference}$ and left DLPFC activity. These results indicate that our moderate exercise under 13% O_2 that elicits apparent hypoxia lowers task-related activities in the DLPFC and deteriorates executive function. We found for the first time that during moderate exercise under 13% O_2, resultant cerebral hypoxia associated reduced neuronal activities of the DLPFC, might be attributed to cognitive fatigue. Thus, moderate exercise under hypoxic conditions could be an experimental model for addressing the neural mechanism underlying exercise-induced central fatigue.

Disclosures: **G. Ochi:** None. **K. Hyodo:** None. **K. Suwabe:** None. **H. Soya:** None.

Poster

747. Plasticity and Disorders of Executive Function

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 747.14/KKK62

Topic: H.02. Human Cognition and Behavior

Title: Executive functioning in girls with posttraumatic stress disorder secondary to sexual abuse

Authors: *S. PRECIADO MERCADO, A. SANZ MARTIN;
Inst. De Neurociencias, Guadalajara, Mexico

Abstract: Child sexual abuse is a stressing event which is commonly associated with anatomical and functional changes in prefrontal cortex, impulsive and maladaptive behaviors and psychopathology like Posttraumatic Stress Disorder (PTSD). The aim of this study was to characterize the performance in several task that asses the executive functioning in girls with PTSD secondary to child sexual abuse. Forty 12-15 years old girls were evaluated: 20 with PTSD secondary to sexual abuse (PTSD) and 20 healthy girls without abuse history or psychopathology (CO). The participants were paired according to their socioeconomic status, their IQ and scholarship. To determine the presence and intensity of PTSD, the child PTSD sympton scale (CPSS) was applied. The executive funtioning was evaluated with BANFE battery

(Flores, Ostriosky & Lozano, 2014), which is a set of test that allow to asses cognitive proceses related with anterior, orbital and dorsolateral prefrontal areas. In BANFE, the PTSD group showed compared with CO group, lower scores in all indexes (global, anterior, dorsolateral and orbital). Besides, there were significant negative correlations between the intensity of symptoms of PTSD and the BANFE indexes. These results indicate that girls with PTSD have a generalized prefrontal impariment, wich, probably is related with the history of child abuse. This prefrontal disfunction could predispose them to develop psychopathology and maladaptive behaviors.

Disclosures: S. Preciado Mercado: None. **A. Sanz Martin:** None.

Poster

747. Plasticity and Disorders of Executive Function

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Program#/Poster#: 747.15/KKK63

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant R01MH096861

Title: Enhancement of multitasking performance by transcranial alternating current stimulation (tACS)

Authors: *W.-Y. HSU, T. ZANTO, A. GAZZALEY;
UCSF, San Francisco, CA

Abstract: Multitasking is associated with the generation of stimulus-locked theta (4-7 Hz) oscillations arising from prefrontal cortex (PFC), and it has further been shown that transcranial direct current stimulation to PFC improves multitasking performance. However, the causal role of PFC theta oscillations in multitasking abilities has not been established. Here, we investigate if oscillatory theta stimulation via transcranial alternating current stimulation (tACS) may be used to enhance endogenous neural oscillations and improve multitasking performance. To address this, eighty healthy young adults received brief sessions of bilateral PFC theta-tACS while they were engaged in a multitasking challenge, along with the collection of electroencephalography (EEG) data. Participants were separated into four subgroups: rapid anti-phase (anti-phase tACS with a 1-min inter-session interval), slow anti-phase (anti-phase tACS with a 5-min inter-session interval), rapid in-phase (in-phase tACS with a 1-min inter-session interval), and sham (those who received sham tACS in all the sessions as control). Anti- and in-phase refers to the tACS current polarity between bilateral PFC. Importantly, the total amount of stimulation (duration and intensity) was equivalent for all of the stimulation groups. Compared to a sham control group, *only the* rapid-rate group showed enhancement of multitasking over time,

coupled with an after-effect modulation of frontal alpha (8-12 Hz) and posterior beta (13-30 Hz) activities. Across participant regression analyses indicated that those participants with the greatest improvement in multitasking performance also exhibited the greatest increases in frontal alpha and posterior beta oscillations. Interestingly, the slow-rate and in-phase groups did not generate noticeable performance improvements compared to the sham control group. These results suggest tACS effects may cumulate when presented in rapid succession with anti-phase stimulation, thus stressing the significance of delivery protocol for generating positive stimulation-effects using tACS. Moreover, these results indicate frontal theta tACS generates benefits on multitasking performance accompanied by widespread neuroplastic changes across multiple oscillatory frequencies.

Disclosures: W. Hsu: None. **T. Zanto:** None. **A. Gazzaley:** None.

Poster

747. Plasticity and Disorders of Executive Function

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Topic: H.02. Human Cognition and Behavior

Support: INSERM, RBM C11-40

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Institut du Cerveau et de la Moelle Epiniere (ICM) Foundation

Régie Autonome des Transports Parisiens (RATP)

France Parkinson Association

Fondation pour la Recherche Médicale

Title: Proactive inhibitory activity in the subthalamic nucleus is related to akinesia in patients with Parkinson's disease.

Authors: *M. ALBARES[1,2,3,4], B. LAU[3], P. LAVIRON[3,5,4], J.-E. LE DOUGET[3], K. LEHONGRE[3,6], S. FERNANDEZ-VIDAL[3,5,6], P. BOULINGUEZ[7], C. KARACHI[2,3], M.-L. WELTER[2,4,3];
[1]INSERM UMR 1127, Paris, France; [2]Assistance Publique-Hôpitaux de Paris AP-HP, Groupe Hospitalier Pitié Salpêtrière, départements de Neurologie et de Neurochirurgie, Paris, France; [3]Sorbonne Universités, UPMC Univ. Paris 06, UMR S 1127, CNRS UMR 7225, ICM, F-75013,

Paris, France; [4]plateforme PANAM, ICM, Paris, France; [5]CENIR, Paris, France; [6]plateforme STIM, ICM, Paris, France; [7]Hôpital Neurologique Pierre Wertheimer, Hospices Civils de Lyon, Univ. Lyon 1, Villeurbanne, CNRS, UMR5229, Ctr. de Neurosci. Cognitive, lyon, France

Abstract: Parkinson's disease (PD) results from the degeneration of the dopaminergic neurons of the sutbstantia nigra pars reticulata. In consequence, the neuronal activity of the basal ganglia circuitry is highly modified with, in particular, an hyperactivity with increased bursts and oscillations in the subthalamic nucleus (STN). The role of these neuronal activity changes of the STN in the occurrence of clinical motor signs is still unknown. Akinesia with slowness and/or reduced amplitude of the movement is one of the cardinal parkinsonian motor signs. Recently, it has been proposed that akinesia may result from an excessive proactive inhibition of the action initiation, a cognitive process known to inhibit undesired or inappropriate prepotent responses to stimuli. Brain networks involve in this mechanism includes, at least, the supplementary motor area, the precuneus and the STN. In this study, we aim to further understand the role of the STN in this proactive inhibitory control. We recorded STN neuronal activity in 17 PD patients during (single unit recordings), and after (local field potentials recordings-LFP) surgery for deep brain stimulation, while subjects performed a cognitive task that involved the proactive inhibitory control. The increased movement initiation latency, induced by the proactive inhibitory control associated with STN neuronal activity changes (30/100 neurons change their activity). The majority of STN neurons recorded intrasurgically exhibited a tonic activity that appeared before the conditioning stimuli and correlated with task performances. STN-LFP recorded post-surgically were modulated in the alpha and beta bands during proactive inhibitory control. Our data suggest that the STN influences the proactive inhibitory control, with PD patients being maintained in an anticipated inhibition, even though the situation did not require action restraint. These data highlight the crucial role of the STN in motor control and shed light on the complex function of this structure in the motor and executive behavior in humans.

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Poster

747. Plasticity and Disorders of Executive Function

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 747.17/KKK65

Topic: H.02. Human Cognition and Behavior

Support: NYU Deans Undergraduate Research Fund (DURF)

Title: A randomized controlled study examining 13 minutes of daily meditation training on attention, mood and the emotional response to acute stress

Authors: *A. C. MCHALE, J. C. BASSO, V. J. ENDE, W. A. SUZUKI;
Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: Meditation is an ancient practice that stems from Buddhist and Hindu cultures. A major purpose of meditation is to focus the mind, clearing it of the propensity towards wandering or unfocused thought. The scientific literature has examined mainly three types of meditation including focused attention, open monitoring and loving kindness. Recent research assessing the capacity of meditation to change the brain has shown that meditation decreases stress, improves mood, boosts cognitive functioning, increases the brain's functional connectivity, and enhances alpha and theta power—brain states associated with relaxation. Few studies, however, have examined the effects of brief, daily meditation on cognitive functions in a randomized controlled design. To address this hole in the literature, we sought to examine whether only 13 minutes of a daily meditation practice in healthy adults could improve cognitive functioning and mood as well as the emotional response to an acute stressor. In this randomized controlled study, healthy adults (18 to 45 years of age) with little to no experience meditating underwent either 8 weeks of a daily, 13-minute meditation practice (n=16) or listened to a Radiolab podcast (control) (n=15). The meditation practice we utilized involved a simple step-by-step guide in various meditative breathing exercises and a single full-body scan. Before and after the intervention, subjects completed a battery of neuropsychological tasks as well as several self-reported mood questionnaires. At the end of the 8 weeks, subjects also completed the Trier Social Stress Test (TSST), a test combining mental arithmetic with social evaluation that served as an acute stressor. Meditation significantly enhanced attention as assessed by the Stroop Test (Time x Group: $F(1,28)=5.025$, $p=0.033$, partial $\eta 2=0.152$) and decreased anxiety as assessed by the Beck Anxiety Inventory (Time x Group: $F(1,29)=4.758$, $p=0.037$, partial $\eta 2=0.141$). Additionally, compared to controls, meditators showed a significantly diminished emotional response to an acute stressor (Time x Group: $F(3.316,92.843)=2.683$, $p=0.046$, partial $\eta 2=0.087$). These results suggest that even brief, daily meditation training in healthy adults can improve attention and mood as well as decrease emotional activation to an acute stress.

Disclosures: **A.C. McHale:** None. **J.C. Basso:** None. **V.J. Ende:** None. **W.A. Suzuki:** None.

Poster

747. Plasticity and Disorders of Executive Function

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Program#/Poster#: 747.18/KKK66

Topic: H.02. Human Cognition and Behavior

Support: New York University internal funds

In-kind donation from Swerve Fitness

Title: A randomized controlled study examining the effects of enhancing fitness on mood and recognition memory in healthy adults

Authors: ***J. C. BASSO**[1], T. R. LEE[1], C. CROSTA[1], N. PAYNE[1], D. KADAKIA[1], R. TRIVEDI[1], T. WANG[2], W. A. SUZUKI[1];
[1]Ctr. for Neural Sci., New York Univ., New York, NY; [2]Grad. Sch. of Biomed. Sci., Mount Sinai, New York, NY

Abstract: Chronic exercise in rodents causes a variety of plasticity-related changes in the hippocampus, a brain region integral for learning and memory. For example, voluntary wheel running increases levels of neurotrophic factors, neuronal proliferation and survival, long-term potentiation, spinogenesis, angiogenesis and gliogenesis. These changes lead to exercise-induced enhancements in spatial learning, pattern separation, and object recognition memory, as well as improvements in anxiety- and depressive-like behaviors. While many studies have examined the effects of exercise in children and the elderly, surprisingly few studies have examined the effects of chronic exercise on hippocampal-dependent learning and memory in non-aged adults. To address this gap in the literature, we conducted a randomized-controlled study to determine whether engagement in long-term aerobic exercise would result in improvements in hippocampal-dependent behaviors including cognitive functioning and mood. In healthy, previously sedentary and low-fit middle-aged adults (35-59 years of age), recognition memory, pattern separation, episodic memory, spatial navigation ability, and mood as gauged through self-reported questionnaires, were assessed before and after 3 months of indoor cycling involving both high-intensity sprints and endurance training (n=18) or video gaming (control) (n=13). Additionally, changes in cardiopulmonary fitness (VO_2 max) were captured as we hypothesized that those individuals with the greatest changes in fitness would show the largest improvements in cognition and mood. Our results parallel findings in the rodent literature. Both recognition memory as tested with the Mnemonic Similarity Task (Time X Group: $F(1,19)=7.018$, $p=0.016$, partial $\eta 2=0.270$) and general positive affect as measured with the Positive and Negative Affect Scale (Time X Group: $F(1,13)=8.370$, $p=0.013$, partial $\eta 2=0.392$) improved as a result of the exercise intervention. Additionally, those individuals who made the largest gains in cardiopulmonary fitness received the greatest benefits in terms of mood improvement ($R^2=0.686$, $p=0.005$). These results suggest that similar to rodents, chronic exercise produces improvements in both mood and behaviors associated with the hippocampus and associated structures. We hypothesize that a critical factor underlying this outcome was the high-intensity exercise regimen that might have resulted in high levels of neurogenesis in our healthy non-aged adults.

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Poster

747. Plasticity and Disorders of Executive Function

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Program#/Poster#: 747.19/KKK67

Topic: H.02. Human Cognition and Behavior

Support: NYU Dean's Undergraduate Research Grant

Title: A randomized controlled study comparing the effects of continuous aerobic exercise, high-intensity interval training, and walking on mood and cognition

Authors: *C. CROSTA, J. C. BASSO, M. RASKIN, P. SEHMBEY, W. A. SUZUKI;
Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: Acute aerobic exercise causes significant improvements in mood and cognitive functioning. High-intensity interval training (HIIT), an exercise regimen comprised of brief bursts of vigorous aerobic exercise interspersed with low intensity recovery periods, has significant physiological advantages over continuous aerobic exercise (CAE). These advantages include significantly higher average heart rates, increased levels of circulating catecholamines, cortisol and growth hormones, increased lactate levels, and enhanced blood glucose utilization. While the enhanced cardiopulmonary benefits of HIIT are well known, it is unknown if HIIT has superior mood and cognitive benefits relative to CAE. As HIIT produces heightened physiological responses over CAE, we hypothesized that an acute bout of HIIT will also produce greater mood and cognitive enhancements over an acute bout of CAE. In this randomized controlled study, we examined whether the enhanced physical benefits of HIIT are accompanied by enhanced cognitive and mood benefits at the behavioral level. Healthy, active adults (ages 18-26) conducted a variety of mood questionnaires and neuropsychological tasks both before and after either a 44-minute session of HIIT (n=13), CAE (n=13) or walking (control) (n=15). All exercise regimens produced significant decreases in total mood disturbance as measured by the Profile of Mood States (Time: $F(1,30)=6.490$, $p=0.016$, partial $\eta2=0.178$), general negative affect as measured by the Positive and Negative Affect Scale (Time: $F(1,30)=18.429$, $p=0.000$, partial $\eta2=0.381$), and anxiety as measured by the Beck Anxiety Inventory (Time: $F(1,30)=5.446$, $p=0.027$, partial $\eta2=0.154$) as well as increases in happiness (Time: $F(1,30)=4.341$, $p=0.046$, partial $\eta2=0.126$) and empathy (Time: $F(1,30)=4.685$, $p=0.039$, partial $\eta2=0.165$) as measured by the fantasy subscale of the interpersonal reactivity index. Additionally, though state-trait anxiety decreased in all groups (Time: $F(1,30)=7.427$, $p=0.011$, partial $\eta2=0.198$), this effect differed between groups (Time X Group: $F(2,30)=4.232$, $p=0.024$, partial $\eta2=0.220$) with the walking intervention showing the strongest effect and the intensity of the workout (based on percentage of VO_2 max) positively correlating with change in state-trait anxiety score ($R^2=0.210$, $p=0.011$). These results suggest that in healthy young adults, low-intensity exercise may

be the best acute workout regimen to enhance mood. We hypothesize that this effect may be due to the fact that compared to the walking group, CAE and HIIT groups found their workout significantly more fatiguing (Subject Exercise Experience Scale; $F(2,40)=4.555$, $p=0.017$).

Disclosures: **C. Crosta:** None. **J.C. Basso:** None. **M. Raskin:** None. **P. Sehmbey:** None. **W.A. Suzuki:** None.

Poster

747. Plasticity and Disorders of Executive Function

Location: Halls B-H

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Program#/Poster#: 747.20/KKK68

Topic: H.02. Human Cognition and Behavior

Support: NIH, R01-AA016624

SDSU Start-up Funds

Title: Binge drinking is associated with reduced EEG-based indices of emotional processing and memory retrieval

Authors: ***S. HUANG**[1], L. A. HOLCOMB[1], S. M. CRUZ[1], K. MARINKOVIC[1,2];
[1]Psychology, San Diego State Univ., San Diego, CA; [2]Radiology, UCSD, San Diego, CA

Abstract: There is a considerable amount of research showing dampening effects of chronic alcohol use on emotional processing but few studies have addressed this topic in individuals with a history of binge drinking. The present study examined the processing of emotional pictures and subsequent memory retrieval as a function of binge drinking. Fifty young, healthy individuals (age = 23.5 ± 3.5 yrs, 22 males) participated in both sessions of the study. Twenty four participants reported drinking heavily with 11.4 ± 7.5 binging episodes and 4.4 ± 3.0 alcohol-induced blackouts in the last 6 months and twenty six participants were light social drinkers. The binge drinking group reported higher depression, anxiety, and impulsivity than the light drinking group. In the first session, participants rated valence of unpleasant, neutral, pleasant, and erotic pictures from the International Affective Picture System. An unexpected recognition task was administered 48 hours later. Scalp EEG was recorded from 64 channels and analyzed in time-domain as event-related potentials (ERPs) and in time-frequency domain with Morlet wavelets. Both groups gave expected ratings of emotional valence. During the rating task, erotic pictures elicited the greatest event-related theta power (4-7Hz) overall. The difference in theta power between all emotional and neutral pictures was apparent in the light drinking group and blunted in binge drinkers. Furthermore, the early differential sensitivity to negative, positive, and neutral

images in 200-300 ms was present only in light drinkers. ERP analysis indicated that erotic pictures elicited the greatest positivity within 400-650ms time window, followed by negative and positive pictures, with the smallest positivity to neutral pictures. During recognition, emotional pictures were remembered better than neutral pictures. Greater theta power was evoked by the remembered pictures as compared to forgotten pictures only for the light drinking group, consistent with theta involvement in memory processes. Similarly, ERP analysis suggested that the remembered pictures elicited higher amplitude of the positive potential in 550-800 ms, which was absent for the binge drinkers. These findings indicate that binge drinking is associated with reduced processing of emotional information and impaired memory retrieval. Even though behavioral measures did not differ between the two groups, theta oscillations were particularly sensitive to these effects in concordance with the theories suggesting that may be an index of affective processing and memory retrieval.

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Poster

748. Computational Models of Reward and Decision Making

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Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

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Topic: H.02. Human Cognition and Behavior

Support: R01DA038063

R01MH104251

Title: Dynamic quantification of the subjective cost of self-control

Authors: ***C. M. RAIO**[1], P. GLIMCHER[1,2];
[1]New York Univ., New York, NY; [2]Inst. for the Interdisciplinary Study of Decision Making, New York, NY

Abstract: Emerging decision-making research suggests that choosing to forego tempting, but suboptimal, rewards in the service of achieving greater overall outcomes (i.e., exercising self-control) may be intrinsically costly to individuals. These ‘control costs’ are thought to stem from the limited cognitive resources available to support the cognitive demands of exercising self-control. However, an empirical quantification of these costs in humans has not yet been accomplished. We employ a novel decision-making task driven by economic theory to quantify idiosyncratic self-control costs in healthy individuals that are currently on a diet to lose or

maintain weight. Participants first rated a series of food items on level of health, taste and temptation. These subjective ratings allowed us to identify a high and low temptation food for each individual. Participants were asked to remain in the experiment room with the high temptation food for a period of time without eating it in order to acquire a monetary bonus. Critically, before exposure and at regular intervals after exposure, participants reported their willingness to pay (from a $10 endowment) to remove the high tempting good and replace it with the low tempting good, effectively revealing their subjective cost of exercising self-control. Bids were realized using a standard economic auction procedure (Becker-DeGroot-Marschak method) at a fixed low hazard rate. Bidding data revealed that, overall, individuals were willing to pay a monetary cost to eliminate both future and current temptation from their environment. On average, bids to remove the tempting food increased over time, suggesting that the perceived cost of exercising self-control grew the longer participants were exposed to the tempting food. We found that subjective control costs were strongly modulated by how long individuals had successfully maintained their diets. Specifically, a negative correlation emerged between the number of weeks on the diet and average bids across the session. Our results provide novel evidence that self-control costs can be numerically quantified in humans and that these costs increase as exposure to temptation (i.e., self-control duration) increases. These findings may open new avenues of research investigating how manipulating subjective self-control costs can promote more adaptive decision-making in the presence of rewards.

Disclosures: C.M. Raio: None. **P. Glimcher:** None.

Poster

748. Computational Models of Reward and Decision Making

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Program#/Poster#: 748.02/KKK70

Topic: H.02. Human Cognition and Behavior

Support: The John Templeton Foundation, Grant # 36751

The John Templeton Foundation, Grant # 57876

Title: Adaptive task representations in context-based decision making

Authors: *O. LOSITSKY[1], R. C. WILSON[2], M. SHVARTSMAN[1], J. D. COHEN[1];
[1]Princeton Neurosci. Inst., Princeton Univ., Princeton, NJ; [2]Psychology, Univ. of Arizona, Tucson, AZ

Abstract: Contextual information (such as location or current goal) often determines which actions are rewarding at a given moment. For instance, a sudden downpour should trigger opening an umbrella outdoors, but should elicit a different response (such as checking for fire) indoors. How do we develop action priors that are general enough to apply to novel contexts, yet specific enough to maximize reward for a given context?
Here, we investigate how context influences priors for decisions in the simplest task that can be used to study context effects, the AX-continuous performance test (AX-CPT). In this task, a cue (A or B) signals the correct response for a probe (X or Y), which appears two seconds later. An agent seeking to maximize immediate reward rate should use the cue to adapt their starting point for the decision on the probe, whenever the cue provides information about the most likely response. For example, when AX trials (e.g., requiring a left response) are more frequent than AY trials (requiring a right response), participants can respond faster on average if they prepare a left response when the A cue is presented.
To test how the cue affects the prior for the decision on the probe, we built a context-dependent drift diffusion model of behavior in this task, with different starting point and threshold parameters for A and B trials. We then varied the frequency of the probe (X/Y) given the cue (A/B) across participants. Fitting our model to experimental data showed that participants adopted cue-specific priors when the two cues were associated with sufficiently different response probabilities. In contrast, when A and B predicted sufficiently similar responses, participants adopted priors that were indistinguishable for A and B. Furthermore, the difference between the estimated A and B priors was predicted by the improvement in reward rate that could be gained by differentiating contexts.
However, for the case in which the estimated A and B priors were similar, it was not clear whether this reflected the use of a single prior or two distinct priors with similar values. To test this, we changed the frequencies of BX and BY trials mid-way through the experiment. We predicted that, for participants who started out with similar A and B response probabilities, changing the frequencies for B trials would also change their prior on A trials. In contrast, participants who started out with different responses probabilities for the two cues should not show this effect. We report evidence in favor of these predictions.
Our data suggest that humans prefer to adopt simpler, more general priors for decisions, sharing information between contexts, unless the cost in performance becomes too high.

Disclosures: **O. Lositsky:** None. **R.C. Wilson:** None. **M. Shvartsman:** None. **J.D. Cohen:** None.

Poster

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Topic: H.02. Human Cognition and Behavior

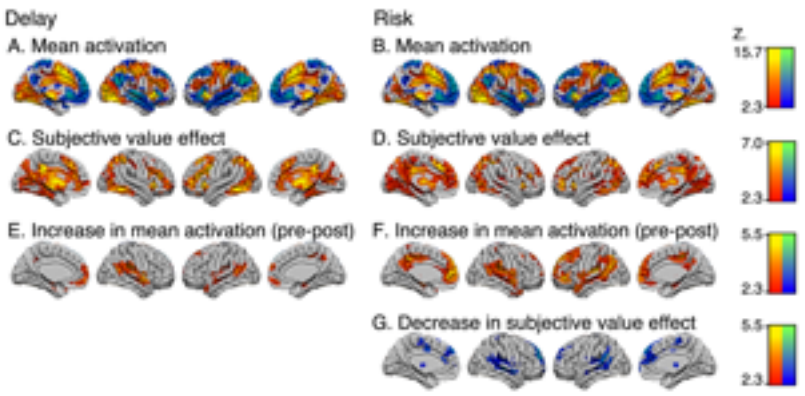
Support: NCI R01-CA170297

NCI R35-CA197461

Title: Cognitive training, the brain, and decision making

Authors: ***M. K. CAULFIELD**[1], J. KABLE[1], M. FALCONE[2], M. MCCONNELL[2], L. BERNARDO[2], T. PARTHASARATHIA[2], N. COOPER[2], R. ASHARE[2], J. AUDRAIN-MCGOVERN[2], R. HORNIK[2], P. DIEFENBACH[3], F. LEE[3], C. LERMAN[2];
[1]Psychology, [2]Univ. of Pennsylvania, Philadelphia, PA; [3]Drexel Univ. Westphal Col. of Media Arts & Design, Philadelphia, PA

Abstract: Increased brain activity in cognitive control circuits can shift choice behavior away from immediate and risky rewards. Motivated by this evidence, we tested whether training of working memory and executive cognitive function could influence choice behavior and brain responses. In this randomized controlled clinical trial, 128 young adults participated in 10 weeks of training with either a commercial web-based cognitive training program or web-based video games (with no working memory or adaptive training component). Pre- and post-training, participants completed cognitive assessments and functional magnetic resonance imaging (fMRI) during performance of validated decision-making tasks: delay discounting (choices between smaller rewards now vs. larger rewards in the future) and risk sensitivity (choices between larger riskier rewards vs. smaller certain rewards). Contrary to our hypothesis, we found no evidence that commercial cognitive training influences neural activity during decision-making, nor did we find effects of cognitive training on measures of delay discounting or risk sensitivity. Participants in the commercial training condition did improve with experience and practice on the specific tasks they performed during training, but this improvement did not represent growth in cognitive abilities, as participants in both conditions showed similar improvement on standardized cognitive measures over time. Though performance on the specific tasks that comprise commercial cognitive training regimens can improve with practice, commercial adaptive cognitive training in healthy young adults appears to have no generalized effects on neural activity, choice behavior, or cognition.



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Poster

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Program#/Poster#: 748.04/LLL2

Topic: H.02. Human Cognition and Behavior

Support: NISEFY16BAR019

Title: Using cognitive biomarkers to assist human performance on attention-related tasks

Authors: ***M. D. RIEDY**;
71000 Res. and Applied Sci., SPAWAR Systems Ctr. Atlantic, North Charleston, SC

Abstract: Throughout the Navy and the other armed forces of the United States, human system operators must maintain vigilant attention across a wide variety of system interfaces. They must be constantly prepared to rapidly respond, often with life or death consequences. Importantly, the neurobiological mechanisms that support the ability to maintain focused attention have physical limits. As system operators in demanding environments approach these limits, their task performance will suffer. In order to mitigate the unavoidable negative impact of these limitations on task performance to the greatest extent possible, we must first possess a thorough understanding of the fundamental neural processes, and their related biological indicators, which underlie the maintenance of sustained focused attention. A robust population template of the

biological indicators of focused-attention and performance-related cognitive states in humans has been developed and continues to be refined by the many excellent research teams in this exciting field. This presents an opportunity to leverage this knowledge to supplement the biological limitations of system operators via the integration of computerized attention and decision-making assistance. In order to contribute to the understanding of the basic science of these processes in our own lab, we collect and analyze electroencephalography (EEG) voltage data, heart rate, eye-gaze location and duration, saccade rate, pupillometric dynamics, and other non-invasive biological measurements in the context of visual search and other attentionally-demanding behavioral tasks. Current findings are consistent with the extant literature regarding biological indicators corresponding to the cognitive states of high versus low cognitive load. As we continue to reproduce well-accepted findings in these and other paradigms, we pivot towards the utilization of the most reliable of these measures in the development of an integrated human and computer system, or brain-computer interface, which aims to ameliorate the physical limitations of sustained focused attention, as evidenced in improvements in reaction time and other performance-related parameters.

Disclosures: M.D. Riedy: A. Employment/Salary (full or part-time): US Dept. of Defense, SPAWAR SSC Atlantic.

Poster

748. Computational Models of Reward and Decision Making

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Topic: H.02. Human Cognition and Behavior

Support: BMBF; Grant numbers: FKZ 01GQ0913, FKZ 01GQ1313

Title: Electrophysiological dissociation of learning-related uncertainty- and surprise signals

Authors: *R. BRUCKNER[1], M. R. NASSAR[2], H. R. HEEKEREN[1], B. EPPINGER[3]; [1]Biol. Psychology and Cognitive Neurosci., Freie Univ. Berlin, Berlin, Germany; [2]Dept. of Cognitive, Linguistic, and Psychological Sci., Brown Univ., Providence, RI; [3]TU Dresden, Dresden, Germany

Abstract: Adaptive behavior requires the computation of expected future outcomes. Especially in dynamic environments, newly incoming information should be used to adapt beliefs to changing environmental states (e.g., changing stock prices). Previous work suggests that human participants are capable of adjusting their learning rate (i.e., how much to learn from environmental feedback) according to the uncertainty and surprise associated with an outcome

(e.g., Nassar et al. Nat. Commun., in press). Uncertainty reflects the reliability of internal beliefs and is highest immediately after a change in the environment is detected. Surprise reflects the likelihood of a change in outcome contingencies. In this study we were interested in electrophysiological (EEG) correlates of uncertainty- and surprise driven learning. In a predictive inference task younger adults (N=31) had to infer the position of an invisible cannon to catch as many cannonballs as possible. We compared this "learning" condition with two control conditions in which participants did not have to infer the target of the cannon but either followed the visible cannon or "collected" cannonballs by following the outcomes. These control conditions allow a dissociation between learning-related activity and activity that is associated with sensory processing in the task. Behavioral data were analyzed using computational modeling and regression analyses. Consistent with earlier behavioral findings, in the learning condition, we found that participants dynamically adjusted their learning rates in response to uncertainty and surprise. In contrast, in the control conditions, subjects showed constant learning rates that were unaffected by surprise and uncertainty. Preliminary EGG analyses suggest that parietal uncertainty-related learning signals arise before outcomes are presented (~150 ms) whereas surprise is associated with a positive medial prefrontal deflection peaking at 400 ms after the presentation of outcomes. Taken together, our results suggest that electrophysiological correlates of uncertainty- and surprise-related influences on learning are temporally and spatially dissociable.

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Poster

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Topic: H.02. Human Cognition and Behavior

Support: McKnight Foundation Memory and Cognitive Disorders Award

Title: Examining the role of memory retrieval in value-based decision making

Authors: *A. BAKKOUR[1], Y. H. R. KANG[2], M. N. SHADLEN[2,3,4,5], D. SHOHAMY[1,3,4];
[1]Dept. of Psychology, [2]Dept. of Neurosci., [3]Zuckerman Mind, Brain, Behavior Inst., [4]Kavli Inst. for Brain Sci., Columbia Univ., New York, NY; [5]Howard Hughes Med. Inst., New York, NY

Abstract: The speed and accuracy of many decisions conform to regularities of bounded evidence accumulation. Such models have proven successful for understanding perceptual

decisions made from dynamic sensory input, where integration of independent samples of evidence is normative. However, the same framework applies to value-based decisions that do not implicate integration. Here we test the hypothesis that the sequential character of such tasks involves the retrieval of evidence from memory. In the current study, subjects were scanned with fMRI while performing a value-based decision task, and a perceptual decision task for comparison. The value-based decision task required subjects to make a series of choices between two foods that varied in subjective value ($\Delta V=V_i - V_j$) as assessed prior to scanning. The perceptual task required subjects to discriminate the predominant color in a stochastic random dot display comprised of yellow and blue dots. Difficulty was controlled by the probability that a dot color was yellow or blue and this "color coherence" was chosen randomly on each trial. The ΔV (for value decision task) and color coherence (for the perceptual decision task) were entered as modulated regressors in the imaging analysis and we compared the magnitude of the effects of ΔV and color coherence on BOLD activity. Subjects chose the higher-value food more often and their reaction times decreased as a function of $|\Delta V|$. Similarly, subjects accurately indicated the predominant color in the color dots display more often and their reaction time decreased as a function of the color difficulty. Thus, behavior indicated a good match between the value-based and perceptual decision tasks. FMRI data revealed that the two kinds of decisions, when evaluated separately, were associated with activity in distinct brain regions. Specifically, for value-based decisions, modulation of whole-brain BOLD by $|\Delta V|$ revealed activation in cingulate cortex. By contrast, modulation of whole-brain BOLD by color coherence during perceptual decisions was associated with BOLD activity in lateral parietal cortex. Interestingly, a direct contrast of the two tasks revealed greater BOLD activity in an ROI in the hippocampus, a region known for its role in memory retrieval. This result indicates that the relationship between ΔV and BOLD in the hippocampus during food choice was stronger than the relationship between color coherence and BOLD during perceptual decisions. Value-based decisions were associated with greater hippocampal activity, supporting the hypothesis that memory-related brain regions contribute more to value-based than perceptual decisions.

Disclosures: **A. Bakkour:** None. **Y.H.R. Kang:** None. **M.N. Shadlen:** None. **D. Shohamy:** None.

Poster

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Topic: H.02. Human Cognition and Behavior

Support: ARC DECRA 140100350

University of Melbourne Faculty of Business and Economics Strategic Initiatives Grant

Title: Caloric primary rewards decrease temporal persistence

Authors: *B. J. FUNG[1], S. BODE[1], C. MURAWSKI[2];
[1]Sch. of Psychological Sci., [2]Dept. of Finance, The Univ. of Melbourne, Melbourne, Australia

Abstract: Intertemporal decisions often involve a tradeoff between delayed rewards and the opportunity costs associated with waiting for those rewards. Thus, optimal intertemporal decision making critically relies on the accurate representation of these temporal opportunity costs. Previous research has suggested that differences in physiological state (e.g. the consumption of caloric rewards) can influence time perception, which may, in turn, alter subjective temporal opportunity costs. Similarly, the energy budget rule in risk-sensitive foraging theory predicts that positive energy balance will result in aversion to uncertain delays. In this study, we investigated whether the consumption of caloric primary rewards could alter subjective temporal opportunity costs and temporal persistence during reward-seeking.

Fifty fasted participants engaged in a temporal persistence task, during which they received either a caloric drink (aspartame and maltodextrin), or water. In this paradigm, participants waited for a large monetary reward (15 cents) delivered at an uncertain time, but at any time could quit waiting in favour of a smaller monetary reward (1 cent). After either reward was received, this process repeated until a 5 minute block elapsed. Thus, this task constituted a reward-maximization problem, akin to foraging. In each block of the task, delivery times of the larger monetary reward were drawn from either a uniform distribution, or a heavy-tailed distribution, which had a higher maximum reward rate. We used survival analysis to estimate participants' probability of quitting conditional on which distribution the delays were drawn from, and conditional on which liquid they consumed.

We found that participants had a higher probability of quitting when rewards were drawn from the distribution with the higher reward rate. This replicates previous findings (McGuire, & Kable, 2012), and suggests that individuals' estimates of opportunity cost are influenced by the reward rate of the environment. Furthermore, we found that participants who consumed the caloric drink had a higher probability of quitting than those who consumed water. This suggests that caloric primary rewards alter perceived temporal opportunity costs and affect temporal persistence, in accordance with the energy budget rule. Our results suggest that both higher monetary reward rates and physiologically relevant rewards can increase impulsive behaviour. This may be especially relevant for disorders such as binge eating disorder, as well as for intertemporal choices in general.

Disclosures: **B.J. Fung:** None. **S. Bode:** None. **C. Murawski:** None.

Poster

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Topic: H.02. Human Cognition and Behavior

Title: The cost of aggression: predicting behavior using a value-based discounting model

Authors: *H. M. DORFMAN, E. H. PATZELT, M. PRATER-FAHEY, J. BUCKHOLTZ; Harvard Univ., Cambridge, MA

Abstract: Traditional models of aggressive behavior often focus on failures in response inhibition. However, this framework cannot account for instances of instrumental aggression. Inspired by economic models of intertemporal discounting, and previous work in moral decision-making (Shenhav & Greene, 2010; Crockett, et al., 2014), we hypothesized that, given the choice between two rewards, subjects would discount the value of the larger reward choice if it involved an aggressive action, and that individual variability in the willingness to aggress would be driven by differences in how strongly subjects weigh the intrinsic cost of harming another person. We tested this hypothesis in a sample of all-male community volunteers using a noel behavioral paradigm. We found large interindividual variability in the willingness to aggress. Using a formal computational model, we found that individual variability is in fact well-fit by a scaling parameter in a modified hyperbolic discounting model, and that the variance accounted for by the free parameter in the model is significantly correlated with self-report measures of aggression and psychopathy.

Disclosures: H.M. Dorfman: None. **E.H. Patzelt:** None. **M. Prater-Fahey:** None. **J. Buckholtz:** None.

Poster

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Topic: H.02. Human Cognition and Behavior

Support: NIH/NIDA 1R01DA038063

NIH F32DA039648

Title: Temporal discounting as an individualized computational marker of treatment trajectory for opioid use disorder

Authors: *S. LOPEZ-GUZMAN[1], A. B. KONOVA[1], A. A. URMANCHE[1], J. B. DENNISON[1], S. ROSS[2,3], K. LOUIE[1], J. ROTROSEN[2], P. W. GLIMCHER[1,4];
[1]Ctr. for Neural Sci., NYU, New York, NY; [2]Dept. of Psychiatry, New York Univ. Sch. of Med., New York, NY; [3]Div. of Alcoholism and Drug Abuse, Bellevue Hosp. Ctr., New York, NY; [4]Inst. for the Interdisciplinary Study of Decision Making, New York Univ., New York, NY

Abstract: Objective: Temporal discounting (TD), or the tendency to forfeit future larger rewards in favor of sooner smaller ones, is an increasingly widespread proxy measure of impulsivity in studies of substance use disorder. By employing this model-based approach, we derive a parameter - the discount rate (DR) - that encompasses the degree to which a reward loses its value as a function of time for each individual. Previous studies have indicated that patients with opioid use disorder (OUD) exhibit higher DRs compared to controls and that patients' DRs decrease during briefly measured treatment periods. However, this change in TD has not been successfully linked to the main objective of treatment, a reduction in opioid use towards abstinence. The utility of DRs as a predictor of relevant clinical outcomes in OUD thus remains a pressing open question: Does it predict relapse or recovery? We sought to address this by conducting a longitudinal within-subjects study with repeated measures of DRs in a cohort of patients starting treatment for mild to severe OUD when compared to matched community controls.
Methods: Repeated measures of DRs were performed on a cohort of thirty patients with OUD starting treatment consisting of pharmacological maintenance and psychosocial intervention for a period of up to seven months. We also collected repeated DRs in a group of forty matched controls from the same community. In the TD task participants were asked to choose between an immediate monetary reward ($2, $5 or $15) and a larger reward that came with a delay (ranging from $7 to $66 and from 4 to 150 days). At the end of each session, one of the trials was randomly selected for realization. In our OUD cohort, subjects' drug use was assessed on every session by self-report, urine toxicology results, length of abstinence and adherence to their treatment plan.
Results and conclusions: As previously reported, OUD patients have significantly higher DRs compared to community controls. We also found that both groups were steeper discounters than the traditional college student sample. Our results indicate that in OUD patients DRs are a dynamic function of time in treatment. Interestingly, DRs also correlate with relapse events, peaking when these occur. We conclude that TD, when assessed repeatedly over the course of treatment, could be used as a behavioral signature of a patient's treatment success and potentially serve as a useful predictor of prognosis and treatment adherence for OUD. Current and future efforts include increasing our sample size and extending the finding of the correlation between clinical state and TD to the investigation of the neural substrate of change in TD with treatment in OUD.

Disclosures: **S. Lopez-Guzman:** None. **A.B. Konova:** None. **A.A. Urmanche:** None. **J.B. Dennison:** None. **S. Ross:** None. **K. Louie:** None. **J. Rotrosen:** None. **P.W. Glimcher:** None.

Poster

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Program#/Poster#: 748.10/LLL8

Topic: H.02. Human Cognition and Behavior

Support: Ragnar Soderberg Foundation

Marianne & Marcus Wallenberg Foundation

Title: The effect of acute pain on risky and intertemporal choice

Authors: ***L. KOPPEL**, D. ANDERSSON, I. MORRISON, D. VÄSTFJÄLL, G. TINGHÖG; Linkoping Univ., Linkoeping, Sweden

Abstract: Pain is a highly salient and attention-demanding experience that motivates people to act. We investigated the effect of pain on decision making by delivering acute thermal pain to participants' forearm while they made risky and intertemporal choices involving money. Participants (n = 107) were more risk seeking under pain than in a no-pain control condition when decisions involved monetary gains but not when they involved equivalent monetary losses. Pain also resulted in greater preference for immediate (smaller) over future (larger) monetary rewards. These findings reflect a motivation to offset the aversive, pain-induced state, where monetary rewards become more appealing under pain than under no pain and when delivered sooner rather than later.

Disclosures: **L. Koppel:** None. **D. Andersson:** None. **I. Morrison:** None. **D. Västfjäll:** None. **G. Tinghög:** None.

Poster

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Program#/Poster#: 748.11/LLL9

Topic: H.02. Human Cognition and Behavior

Support: NIH R01DA038063

Title: Stress exposure decreases cooperative behavior

Authors: ***B. LU**[1], C. M. RAIO[1], M. GRUBB[2], G. S. SHIELDS[3], G. M. SLAVICH[4], P. GLIMCHER[1,5];
[1]Ctr. for Neural Sci., New York Univ., New York, NY; [2]Dept. of Psychology, Trinity Col., Hartford, CT; [3]Dept. of Psychology, Univ. of California-Davis, Davis, CA; [4]Dept. of Psychiatry and Biobehavioral Sci., Univ. of California-Los Angeles, Los Angeles, CA; [5]Inst. for the Interdisciplinary Study of Decision Making, New York, NY

Abstract: Although stress exposure is an inevitable part of daily life where decisions that involve risk and uncertainty are often made, reports of stress effects on decisions about uncertainty are equivocal across the literature. Research examining stress effects on these decisions have primarily focused on choices for which outcome probabilities are explicitly known (i.e., risk). However, decisions are often made when the probabilities of different outcomes are unknown (i.e., ambiguity). Here, we used a standard experimental economic paradigm that dissociates attitudes toward risk and ambiguity to assess how both acute and lifetime stress exposure affects economic decisions regarding uncertainty. Thirty-one healthy individuals first completed the decision-making task to characterize attitudes toward risk and ambiguity under non-stressful conditions. Self-reported state anxiety levels were collected using the State Anxiety Inventory before the choice task, and lifetime stress exposure levels was measured using the Stress and Adversity Inventory (STRAIN), a detailed inventory of encountered stressful life events, after the choice task. A week later individuals returned and were randomly assigned to repeat the decision-making task in the presence or absence of acute stress (cold-pressor or matched control task). Saliva was collected throughout each session to assay neuroendocrine markers of stress responses—namely, cortisol and alpha-amylase. Individuals' choice behavior—as measured by the proportion of risky or ambiguous lotteries selected during the choice task—did not change across sessions for either the stress or control condition. Further, choice behavior did not differ between conditions after stress responses were manipulated during session 2, suggesting that exposure to acute stress alone did not influence participants' attitudes toward risk or ambiguity. However, the STRAIN data revealed a negative correlation between two main indices of lifetime stress exposure (i.e., total count and severity of lifetime stressors experienced), and the proportion of ambiguous lotteries selected, indicating

that higher levels of lifetime stress exposure were related to a lower willingness to accept ambiguous lottery offers. A similar association emerged between state anxiety and the proportion of accepted ambiguous lotteries. These effects were selective to decisions about ambiguity, as neither state anxiety nor life adversity was related to risky lottery choices. These findings suggest that higher states of anxiety as well as lifetime stress exposure may confer a lower tolerance for unknown outcomes during economic decision-making.

Disclosures: **B. Lu:** None. **C.M. Raio:** None. **M. Grubb:** None. **G.S. Shields:** None. **G.M. Slavich:** None. **P. Glimcher:** None.

Poster

748. Computational Models of Reward and Decision Making

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Topic: H.02. Human Cognition and Behavior

Support: JST CREST

KAKENHI 26242087

Title: Emotional and symbolic trust priors differently modulate the reward prediction error signal during trust game.

Authors: ***M. HARUNO**[1], C. FRITH[2];
[1]Natl. Inst. of Information and Communication Technol., Osaka, Japan; [2]Wellcome Trust Ctr. for Neuroimaging, London, United Kingdom

Abstract: Previous human imaging studies demonstrated that reward prediction signal also plays a critical role in establishing human cooperation as in other simpler decision making tasks. However, it remains unexplored how different sources of prior information about partner's trustworthiness affect the reward prediction error signal. Here, to address this issue, we conducted functional magnetic resonance imaging (fMRI) experiments of a trust game (n=28), where in each round, a participant as an investor repeatedly (8 trials) selected to keep or share 20 yen with a trustee (who is a computer agent but instructed as a human) and observed whether the trustee returned the courtesy. The participants were instructed to optimize their behavior by trial and error. Importantly, in each of four sessions, different prior information about trustee's trustworthiness was provided at the beginning: (1) no prior, (2) facial expression prior (Oosterhof and Todorov 2008) (3) symbolic prior representing trustee's social value orientation (Haruno and Frith 2010) and (4) both facial and symbolic priors. In each session, participants played the trust

game against 16 trustees (8: 80% return, 8: 20 % return, and priors were correct for 80% of trustees) and the order of the sessions was counterbalanced across participants. We analyzed behavioral data by a standard reinforcement learning model and used the estimated reward prediction error as a regressor for fMRI analysis (at the timing of feedback, SPM12). We found that activity in the ventral striatum and ventral ACC was correlated with reward prediction error ($p < 0.05$ small volume correction) in the no prior session (i.e., (1)), and no reward prediction error signal when symbolic prior was provided (i.e., ((3) and (4)). However, when only facial expression prior was provided, we found stronger reward prediction error activity ($p < 0.05$ small volume correction) in the ventral striatum and ventral ACC than the no prior case. These results demonstrates that emotional and symbolic trust priors modulate reward prediction error signal in the brain in an opposite way, and may suggest the existence of the hierarchical brain system that determines the reliability on the reward prediction error based on the characteristics of available prior information.

Disclosures: M. Haruno: None. **C. Frith:** None.

Poster

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Topic: H.02. Human Cognition and Behavior

Support: KAKENHI(26120732)

Title: Neural mechanisms and computation that mediates value by others' reward for decision making

Authors: *H. FUKUDA[1], N. MA[1], S. SUZUKI[2], N. HARASAWA[1], K. UENO[1], J. L. GARDNER[3], N. ICHINOHE[4], M. HARUNO[5], K. CHENG[1], H. NAKAHARA[1];
[1]RIKEN, BSI, Saitama, Japan; [2]Tohoku Univ., Sendai, Japan; [3]Stanford Univ., Stanford, CA; [4]Natl. Ctr. of Neurol. and Phychiatry, Tokyo, Japan; [5]Natl. Inst. of Information and Communications Technol., Osaka, Japan

Abstract: Our decisions are often guided by one's own reward, but in social setting, also frequently modified by others' reward. Although well known as behavior, little is known about the underlying neural computations: how others' reward takes part in the process of one's own value-based decision-making and what the neural mechanisms are. For a definite examination of these issues, we devised a novel experimental paradigm that allows us a quantitative assessment under value-based decision-making framework and then combined human fMRI with

computational modeling. Our task was composed of three types of trials (standard, other, and bonus). In standard trials, the subjects performed usual value-based decisions between two options, each of which is associated with probabilistic reward outcome. In other and bonus trials, extra reward to others and the self was attached to either of options so that subjects should consider both standard value and extra value in their decision-making. This setting allowed us to quantify the value of others' reward, in comparison to that of bonus, comparing their relative effect on the choice behavior. Using logistic regression in the behavior, we quantified each value (standard, others', and bonus value) and decision value (final value difference between the options including extra values). We found that others' reward modified the decisions, although the effect is weaker than that by bonus in the same face amount. These quantifications also enabled us to analyze BOLD signal to identify neural correlates for each value and decision value. For the brain signal, we found that both others' and bonus value had significant activations in the dorsolateral prefrontal cortices (dlPFC), whereas only others' value had that in the right temporoparietal junction (rTPJ). Both standard value and decision value had significant activations in the ventromedial prefrontal cortex (vmPFC). Our psychophysical interaction analyses further indicated others' value encoded uniquely in rTPJ is processed in dlPFC and then finally integrated in vmPFC for final decisions. Moreover, we are now investigating the neural correlates that have significant contribution to making decisions in favor of others' reward versus self reward gain in our task, in relation to the individual social value orientation. These together show the neural underpinning for how others' reward take part in the neural processing for self value-based decisions.

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Poster

748. Computational Models of Reward and Decision Making

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 748.14/LLL12

Topic: H.02. Human Cognition and Behavior

Support: KAKENHI(26120732)

Title: Neural mechanisms for deciding with predicting others

Authors: ***N. MA**[1], N. HARASAWA[1], K. UENO[2], N. ICHINOHE[4], M. HARUNO[5], K. CHENG[2,3], H. NAKAHARA[1];
[1]Lab. for Integrated Theoretical Neurosci., [2]fMRI Support Unit, [3]Lab. for Cognitive Brain

Mapping, RIKEN, Brain Sci. Inst., Wako, Japan; [4]Dept. of Ultrastructural Res., Natl. Ctr. of Neurol. and Psychiatry, Tokyo, Japan; [5]The Ctr. for Information and Neural Networks, Natl. Inst. of Information and Communications Technol., Osaka, Japan

Abstract: Humans often make decisions, following reward expectation or value. In social life, however, reward expectation often depends on the behavior of another person. In other words, such social decision-making often involves predicting others' mind and construct the prediction-based value, leading to decisions. However, little is known for neurocomputational mechanisms of combining the prediction of others and making decisions using the prediction-based value. We addressed this question by devising a novel behavioral paradigm and using human fMRI in combination with computational-modeling-based analysis. The basic form of our experiment is to do value-based decision making or choose one of two options for probabilistic reward gain. There are three types of trials, main, self and other. Main trials required the subject to decide based on the prediction of others' decisions, in which the subject chooses for oneself, but each option's value varies upon others behavior (others' decisions in their own choices). Self and other trials examined each component in the main trials; subject performed ordinary, one's own value-based decisions (self trial) or plainly predicted others' decisions (other trial). Using these trial types, we aim to construct the computational model of value-based decision-making with the prediction. In behavior, the subject decisions followed the respective value difference, self-value difference in self trial and predicted-others' value difference in other trial. In main trials, the decisions were captured by three types of value difference: predicted-others' value difference for predicting others and further, based on the prediction, two possible self-value differences, wherein one and the other can be major and minor as accounting the subject's decision and their relative contribution varies dependent on the difficulty of predicting the other in each trial. These results were further confirmed by computational models' fit. We are currently collecting and analyzing human fMRI data. Using model-based analysis, in self and other trials, we got significant activations in valuation and social brain networks, such as vmPFC and right TPJ. In main trials, our preliminary results showed differential neural processing of these key value differences, dependent upon their functions and cognitive demands. For instance, we found that the neural processing of the self-value difference being major and minor is different, depends on how difficult to predict others. These together indicate the hierarchical computational mechanisms from predicting to account for others' mind to making self-decisions.

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Poster

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Program#/Poster#: 748.15/LLL13

Topic: H.02. Human Cognition and Behavior

Title: The social influence on explore-exploit decisions

Authors: *H. SADEGHIYEH[1], S. WANG[2], R. C. WILSON[2];
[2]Psychology, [1]Univ. of Arizona, Tucson, AZ

Abstract: The explore-exploit dilemma is a common behavioral dilemma that arises any time we must choose between exploring unknown options for information and exploiting known options for reward. For example, when ordering dinner at a favorite restaurant, do you explore a new item on the menu, or do you exploit the pizza you usually get? While much work has looked at how humans and animals make explore-exploit decisions in isolation, real world explore-exploit decisions are often made in social situations, and whether other people explore or exploit may influence our own choice. Thus, if a table-mate orders the pizza first, you may be more likely to order something else instead. In this work we investigated the effect of social information on explore-exploit decisions in the lab using a social version of our previously published Horizon Task (Wilson et al. JEP:G 2014). In this task, subjects made a series of decisions between two one-armed bandits that paid out rewards from different Gaussian distributions whose means were initially unknown. To give participants some information about the relative value, at the start of each game, participants saw four examples draws from the bandits (either 2 plays from both, or 3 from one and 1 from the other). We also showed them social information in the form of choices that (we led them to believe) were made by another person faced with the same game. Given this social information there are three ways for subjects to behave: to copy what the other person did (herding), to do the opposite (diversification), or to ignore the other person's response completely. Using a model we quantified the strength of these social effects as the *social bonus*, an extra piece of value given to the other person's choice. By computing the social bonus in a number of different conditions we found it to depend crucially on the interaction of two factors: the number of choices that subjects would make in the future (the horizon) and whether the outcome of the other person's choice would ultimately be revealed. When the other person's outcome would remain hidden, subjects had a higher social bonus in long horizon than short one (more herding) that may reflect the fact that making the right choice is harder in long horizon. When the other person's outcome would be revealed, subjects had a more negative social bonus in long horizon than short one (more diversification) which means that subjects preferred to get more information by choosing the opposite option when there were future opportunities to use them. These findings, suggest that people use social information in nontrivial ways to facilitate explore-exploit decisions.

Disclosures: **H. Sadeghiyeh:** None. **S. Wang:** None. **R.C. Wilson:** None.

Poster

748. Computational Models of Reward and Decision Making

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Topic: H.02. Human Cognition and Behavior

Support: DGAPA PAPIIT IN219516 TO AERC

DGAPA PAPIIT IN218316 TO OPG

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Title: Event-related potentials associated to differential processing of healthy and unhealthy food in children

Authors: M. DOMÍNGUEZ-MARÍN[1], T. V. ROMÁN-LÓPEZ[2], J. A. FRANCO-RODRÍGUEZ[2], P. I. CLAVEL-PÉREZ[2], M. MÉNDEZ-DÍAZ[1], A. E. RUIZ-CONTRERAS[2], *O. PROSPERO-GARCIA[1];
[1]UNAM, Mexico, D. F., Mexico; [2]Lab. Neurogenómica Cognitiva, Fac. Psicología, UNAM, D.F., Mexico

Abstract: Children aged 11-12 are developing skills in making decisions. One health problem worldwide is that food industry advertising that targets children has been related to the increase of childhood obesity. In this sense, it is important to know if children are able to detect differences between healthy and unhealthy food, and if they are more likely to modify their decision making of buying when a promotion is available. Healthy children between 11 to 12 years-old participated in this study. Participants solved two main tasks. In one task, subjects had to decide if they buy a dish of healthy or of unhealthy food. In 50% of trials, a promotional article was presented above the dish and 50% of trials, a non-promotional stimulus was displayed. In the second task, subjects observed the same stimuli presented in the first task, but only the non-promotional stimulus was shown, and they had to decide if the dish displayed was healthy or unhealthy. Children decided to buy more frequently than not to buy. Also, unhealthy food was more preferred to buy than not to buy. Promotion had an effect in some subjects who augmented their decision making to buy when a promotion stimulus was available, and this increase was larger for unhealthy than for healthy food. For the discrimination task between healthy and unhealthy food, larger reaction times were observed for unhealthy, but no differences were observed in percentage of correct responses. Also, a larger amplitude of N400

at frontal sites was observed for unhealthy than for healthy food. These results suggest that children are able to discriminate the energetic density of food and some of the children are vulnerable to promotion to buy more frequently unhealthy food. This study was conducted with the support of the following grants: DGAPA PAPIIT IN219516, IN218316, IA207416 to AERC, OPG & MMD, respectively.

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Poster

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Topic: H.02. Human Cognition and Behavior

Title: Information integration in complex decision problems

Authors: ***E. ELDAR**[1,2], G. BAE[1,2], Z. KURTH-NELSON[1,2], P. DAYAN[3], R. J. DOLAN[1,2]; [1]Wellcome Trust Ctr. for Neuroimaging, [2]Max Planck UCL Ctr. for Computat. Psychiatry and Ageing Res., [3]Gatsby Computat. Neurosci. Unit, UCL, London, United Kingdom

Abstract: Making decisions often entails integrating multiple pieces of information. One way to achieve that is to consider each piece of information in turn. However, this strategy will produce poor results when many pieces of information are available and time is limited. In such circumstances, using the potentially faster strategy of processing multiple pieces of information in parallel is likely to be superior. We hypothesized that these two integration strategies would appear as distinct dynamical signatures in the rapid evolution of stimulus representations during deliberation. We recorded magnetoencephalography (MEG) from 38 participants in a novel behavioral task where, on each trial, participants were shown their 'team', comprising four types of 'players' in varying numbers., Participants' task was to predict how many goals this team would score, based on the previously-learned scoring ability of each player type. Across trials, we independently manipulated problem complexity (the number of players and player types in a given team) and time limit (participants had either 1 or 2 seconds to form their prediction). Overall, participants provided more accurate predictions for less complex problems, and when under less time pressure. However, the effect of time on performance was absent in both the simplest and most complex problems. This suggests that for the most complex problems, participants adopted an information integration strategy that was less sensitive to time constraints, for instance, by considering all players simultaneously. In MEG, team compositions

could be decoded during the visual presentation of the team. Decoding was most successful in low complexity problems and primarily reflected the players that were most relevant to forming a decision (i.e., those that score the most goals). Intriguingly, we also found sustained decoding of team composition during the 1-2 s deliberation period when the team was not overtly visible, potentially reflecting a deliberation process. Modeling the relationships between these neural and behavioral data can shed new light on strategies for information integration under time constraints.

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Poster

748. Computational Models of Reward and Decision Making

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Topic: H.02. Human Cognition and Behavior

Support: NSF Grant #1200830

Title: Sprint to the finish: how effort and time interact in movement decisions

Authors: *C. M. HEALY, A. A. AHMED;
Univ. of Colorado, Boulder, CO

Abstract: When asked to walk a mile, would you rather walk up a hill at the beginning of the mile, or near the end? Changing the time higher effort is required may determine which route you prefer. Given that all routes have the same metabolic cost and completion time, will the timing of effort change your preference? Here, we sought to clarify the role of time with respect to effort by comparing subjective preferences across equal objective utilities. If effort does interact with time, then an individual would care about when they invested a given amount of effort. Subjects completed 2 sessions (A and B) composed of a *Familiarization Task* and a *Choice Task*. In each session, subjects generated 14 effort profiles by isometrically pushing against a force transducer. Session A included effort profiles with brief periods of high effort ("hills"), while Session B included effort profiles with brief periods of low effort ("valleys"). During the *Familiarization Task*, subjects sat in front of a computer monitor and were asked to trace each profile with a cursor on the screen. The cursor moved horizontally at a fixed rate, while subjects controlled the vertical position with their pushing force. All profiles were 17 seconds in duration and normalized to each subject's maximum force exerted. Subjects then completed a series of two-alternative forced choices, in which they chose between pairs of effort

profiles experienced previously. Each of the combinations were presented 5 times distributed randomly (455 total). Subjects were asked to choose the profile which they felt was least effortful. In 10% of the trials, subjects had to execute the chosen effort profile. Subject choices for both sessions were fit to time-sensitive and time-neutral models, using different effort metrics (force, rate of force, or a combination) by maximizing the log-likelihood of the model representing the data. Initial results (n=12) suggest that subjects have a strong preference for shorter durations of high effort and have a temporal preference for when high force and changes in force occur within a movement. Nine of 12 subjects showed a preference for the time-sensitive model (3 procrastinator, 6 precrastinator) over the time-neutral model. Six of these 9 subjects' data were better represented by a model that included both force and rate of force. These results suggest that the utility of effort is indeed time-sensitive, with subjects exhibiting idiosyncratic temporal preferences. Additionally, a utility function which includes the integral of force and rate of force can better explain subject preference than force alone.

Disclosures: C.M. Healy: None. **A.A. Ahmed:** None.

Poster

748. Computational Models of Reward and Decision Making

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Program#/Poster#: 748.19/LLL17

Topic: H.02. Human Cognition and Behavior

Title: High-resolution imaging of the basal ganglia in rapid choice behavior

Authors: *M. MULDER[1], J. SCHOUTEN[2], M. KEUKEN[1], B. HOMMEL[2], B. U. FORSTMANN[1,2];
[1]Univ. of Amsterdam, Amsterdam, Netherlands; [2]Leiden Univ., Leiden, Netherlands

Abstract: In our daily lives we are often confronted with situations in which we have to decide quickly. However, deciding too fast might result in incorrect choices. As such, it is important to find a balance between the speed of our choices and the risk of making an incorrect choice. Typically, the balance between the speed and accuracy of fast (two alternative) choices can be quantified by using accumulation-to-bound models, like the diffusion-decision model (DDM). According to the DDM, the decision process is described as an accumulation of sensory evidence toward a decision threshold. It is assumed that, when speeded choices are required, people select a lower decision threshold that results in faster choices, at the cost of more errors. Imaging studies have shown that nuclei of the basal ganglia (BG) play a role in the selection of the appropriate decision threshold to the demands of the environment (Forstmann et al., 2008). However, in addition to a threshold adaptation, effects of speed-stress on non-decision processes

(e.g., motor preparation) are reported as well (e.g. Rinkenauer et al., 2004). Up until now, this change in the non-decision time has been ignored, since the non-decision time is rather unspecified. Whether nuclei of the BG play a role in changes in non-decision time is unclear.In this study participants (n=20) were asked to perform a perceptual choice paradigm while they underwent high-resolution (7T) functional magnetic resonance imaging (fMRI). During the task, participants were cued to respond either fast or accurate. Modeling the behavioral data with the DDM showed that the best fitting model included changes in both the threshold and non-decision time parameter. To investigate the role of the BG, we selected five regions of interest: striatum, globus pallidus interna/externa, subthalamic nucleus and the substantia nigra.These ROIs were segmented using the individual high-resolution anatomical image. Next, we determined the shape of the hemodynamic response function (HRF) for each participant and ROI separately. These HRFs were used to estimate the BOLD response during task instructions. Additionally, we investigated the relationship between changes in BOLD response and changes in the decision threshold and non-decision time parameters. The results show that activation in different nuclei of the BG seem to drive adaptations of both the decision threshold and non-decision time effects, giving rise to the individual differences in rapid choice behavior. This might indicate that, in addition to a threshold selection, effects of speed instructions on (pre) motor responses can occur even before or after the decision process.

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Poster

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Topic: H.02. Human Cognition and Behavior

Support: NSF-NCS Grant 1533623

Title: A bias-variance trade-off that governs individual variability in learning and decision-making.

Authors: ***C. M. GLAZE**[1], J. W. KABLE[2], J. I. GOLD[1];
[1]Neurosci., [2]Psychology, Univ. of Pennsylvania, Philadelphia, PA

Abstract: Humans often make variable decisions when presented repeatedly with the same stimuli. This trial-to-trial decision noise has typically been considered adaptive only when it promotes exploration of new, potentially useful information sources. Here we propose and

present psychophysical support for the idea that even when no new information can be obtained, decision noise can reflect "mental exploration": adaptive updating of uncertain models of the environment that is promoted by noradrenergic modulation in cortical areas linked with cognitive control. A key prediction is that uncertainty about statistical structure governs a fundamental bias-variance trade-off in decision-making between adaptability (complex models that promote learning but also variable choices) and precision (simpler models that resist change and instead provide reliable choices). We investigate this theory in the context of change detection in volatile environments that challenge subjects to distinguish variability coming from a stable state from volatile changes in the underlying state itself. In several previous experiments, subjects on average could adapt to varying rates of volatile changes, but with an average bias towards immediate sensory information, underweighting the history of task data. However, this degree of adaptability varied considerably across subjects, with those who adapted least doing so by erring more on the side of underweighting data history. We show that a biologically plausible particle-filter algorithm that operates in "hypothesis (model) space" can account for these individual differences. The algorithm fit both choice and reaction time data well for each subject with only 1-2 free parameters governing a prior distribution over this space. The fits captured different dynamics for different subjects, ranging from continuous, adaptive updates to a tendency to treat all conditions in a similar, history-independent manner. The algorithm is normative for learning novel statistical structure given a wide enough prior distribution over hypothesis space, and effectively captures the bias-variance trade-off evident in the behavioral data in terms of the precision of the prior. New experimental work includes change detection experiments paired with pupil diameter measurements recently shown by our group to reliably indicate single-unit activity in the locus coeruleus, the noradrenergic brainstem center. Preliminary data suggest links between pupil diameter and novel latent variables from this particle filter algorithm such as "unexpected certainty" in the decision-making process.

Disclosures: **C.M. Glaze:** None. **J.W. Kable:** None. **J.I. Gold:** None.

Poster

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Topic: H.02. Human Cognition and Behavior

Support: Ecole des Neurosciences de Paris graduate program

Title: Hebbian mechanisms for task-set learning

Authors: *F. BOUCHACOURT, S. PALMINTERI, E. KOECHLIN, S. OSTOJIC;
Lab. De Neurosciences Cognitives, Ecole Normale Superieure, Paris, France

Abstract: Depending on environmental demands, humans engaged in a given task are capable to adjust and exploit multiple, concurrent strategies. We define the internal representations of such stimuli-response mappings as “task-sets”. Theoretical research on rule-guided behavior and the interdependence between learning and cognitive control mainly focused on abstract computational models at the functional level. Little is known however about the underlying neural implementation and mechanisms.
We examine a candidate neural mechanism for the implementation and learning of task-sets, based on hebbian synaptic plasticity. The model is composed of two interacting neural circuits. A decision module learns one to one associations between visual stimuli and motor responses, but cannot learn more than one stimuli-response mapping. The activity in this module drives synaptic plasticity in a second neural circuit that learns event statistics on a longer timescale. When this second module detects patterns in stimulus-action associations, an inference bias to the decision module influences successive behavior.
We show that simple unsupervised hebbian learning in the second module is sufficient to learn an implementation of task-sets. Their retrieval in the decision module improves behavioral performance. It accounts for fast contextual switching and corrects for environmental noise. The model predicts abrupt changes in behavioral responses depending on the precise statistics of previous responses. We fitted the model to human behavioral data, and produced predictions for learning facilitation or learning reduction based on the inference bias. The predictions of the model were borne out by the data, and enabled to identify from behavior alone subjects who have learned the task structure, confirming a post-test debriefing. Preliminary results of our model-based fMRI analysis show a correlation between the inference signal and BOLD activity in fronto-parietal network. Within this network, a dorsomedial prefrontal node seems to be preferentially recruited when task sets are recurrent, suggesting that activity in this region may provide a bias to decision circuits when a task-set is retrieved.
These results show that simple hebbian mechanisms and temporal contiguity may parsimoniously explain the learning of complex, rule-based behavior.

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Topic: H.02. Human Cognition and Behavior

Support: James S. McDonnell Foundation Grant

Title: Brain network connectivity underlying decisions between the "lesser of two evils"

Authors: *C. FINNERTY[1], C. HANSON[2], S. HANSON[2];
[1]Psychiatry, Stanford University/Palo Alto Veterans Administrat, Palo Alto, CA; [2]Rutgers Univ., Newark, NJ

Abstract: While brain response during decisions about appetitive stimuli such as food or money is fairly well characterized, it is less well understood how the brain encodes choices about aversive stimuli. One challenge is that signal related to stimulus salience (e.g. intensity) is often collinear with signal related to valence (e.g. aversiveness). Additionally, if brain areas are valence sensitive, then they should “deactivate” or decrease activation relative to baseline in proportion to how aversive a stimulus is, and increase activation proportional to appetitiveness, whereas areas that respond to salience will increase activation for both appetitive and aversive stimuli. Distinguishing salience from valence specific coding thus requires modeling differences in both increases and decreases in BOLD signal in response to both aversive and appetitive stimuli. Additionally, there is a limited understanding of brain response to stimuli that approximate real-world complexity. Here, we compare two groups of 14 participants who made choices between complex, individualized, hypothetical stimuli during fMRI scanning; one group chose amongst aversive stimuli (e.g. illnesses, car accidents) and one chose between pleasant options (e.g., vacations). We predicted that regions such as the insula, amgydala, and striatum code valence and thus would decrease their activation during aversive, but not appetitive choice. We further characterize this response via graph theoretical connectivity analysis to test whether increased/decreased activation in these regions is also accompanied by increases/decrease in number or strength of connections. This also allows us to compare changes in deactive region connectivity across conditions, which circumvents methodological limits in comparing deactivation using GLM analysis. We found that brain response during hypothetical aversive choices involves activations in areas associated with decision making, including the putamen, insula, and anterior cingulate, as well as deactivations valence sensitive regions, including the amygdala, prefrontal cortex, and hippocampus. In contrast, appetitive choices primarily were related to activations above baseline in these same regions. Further, we find shifts in connectivity between active and deactive brain regions based on task context, with greater connectivity among deactive regions during avoidance choices for aversive stimuli compared to approach choices. This difference in brain network response to decision frame suggests that salience and valence both factor into hypothetical choices to approach or avoid appetitive and aversive stimuli.

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Program#/Poster#: 748.23/LLL21

Topic: H.02. Human Cognition and Behavior

Title: Simple economic choice in large choice sets: an investigation of hick's law

Authors: *A. THOMAS[1], I. KRAJBICH[2];
[1]Technische Univ. Berlin, Berlin, Germany; [2]Ohio State Univ., Columbus, OH

Abstract: Objective: We often have to choose from a large number of options (e.g. choosing a yogurt at the supermarket). Hick's Law predicts that the time that we need to make a choice should linearly increase with the logarithm of the number of options. However, this has yet to be tested in economic choice. Moreover, the neural mechanisms underlying this response time phenomenon remain unknown. We explore these questions using an eye-tracking experiment in which hungry subjects choose snack food items from large choice sets of varying size (N: 9-36).
Methods: 51 hungry individuals participated in this experiment, which consisted of choice and rating tasks. During the choice task, subjects faced 200 randomly assembled sets of snack food items (sizes: 9, 16, 25, 36). For each set, subjects were asked to choose the item that they would like to eat most at the end of the experiment. Subjects had as much time as they wanted to make their choice. In the second task, subjects rated each of the 80 food items, which provided independent measures of their values.
Results: We find that subjects' response times do increase as a logarithmic function of choice set size, while their response accuracy remains constant and high, consistent with Hick's Law. In order to shed more light on these findings, we use the choice, response-time, fixation and value data to compare several computational models of the choice process in this class of environments. The results of this comparison show that the attentional drift-diffusion model qualitatively matches subjects' choice and response-time behavior and outperforms the alternative models on several important dimensions.

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Topic: H.02. Human Cognition and Behavior

Title: A model based in brain-computer interface to identify risk situations and support to decision-making

Authors: *R. HÜBNER[1], A. N. S. HÜBNER[2], P. V. O. MIGUEL[4], L. B. R. AYLON[3], G. BARRETO[5];
[1]Computing Dept., Federal Univ. of Technol. - Paraná, Maringá, Brazil; [2]Pharmacol. and Therapeut. Dept., [3]Computer Dept., State Univ. of Maringá, Maringá, Brazil; [4]Tech. High Sch. of Campinas, Campinas, Brazil; [5]State university of Campinas, Campinas, Brazil

Abstract: The purpose of this study is to use a Brain-Computer Interface (BCI) to identify risk situations and assist a person in decision-making. People lives daily with processes like this and such decisions become more critical when involving life-threading. In addition to environmental factors, cognitive process that supports a plan of action and decision-making takes into consideration the ability, the experience and the emotional states of the holder agent. The use of BCI can help identify risk situations through simulations, and propose specific training for the prevention of these cases, however, submitting people to potential damage from these occurrences. These artifacts can still be used for real time monitoring signals related to relaxation ans traditional models of selective attention based on external stimuli such as Steady State Visual Evoked Potential (SSVEP) and P300. Applications in models that use specific stimuli, for example visual or auditory or tactile. The responses of stimulus usually obtained by specif electrodes in BCI, for example, visual stimulus is picked up by electrodes positioned in visual cortex. Four scenarios was created using a computer simulation based on a virtual game “runner” style. A electroencephalography (EEG) equipment non-evasive with 16-channel is used in the experiment and electrodes are spread in regions of the frontal, occipital, parietal and temporal lobe (channels Fp1, Fp2, F7, F3, F4, F8, T7, C3, C4, T8, P7, P3, P4, P8, O1 and O2 of 10-20 location system). The standardization is performed by checking which brain regions of the cortex are active given a particular decision-making in the simulation. Using temporal filters, major artifacts phenomena are excluded from the signal measured, such as eye blink, heartbeat and other that not contribute with the experimentation. Spatial filters are added in the experimentation to determine channels that measure useful data. Each situation class occurred in the simulation has the same time and its events are mapped. The filtered signal are passed by classifiers used to determine which of the four situations are occurring where and each of them has previously correct action response. Thus, given an event occurred in the simulation, the application can aid in the decision to be taken. Related works and preliminary results show that

visual scenarios can be standardized to a BCI application, to classify and use the feedback to decision-making. In this study is being used a more generalized approach through various channels of EEG to identify risk situations. This work if constantly updating and some improvements in the computer simulation, signal filters and classifiers are still necessary.

Disclosures: R. Hübner: None. **A.N.S. Hübner:** None. **P.V.O. Miguel:** None. **L.B.R. Aylon:** None. **G. Barreto:** None.

Poster

748. Computational Models of Reward and Decision Making

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 748.25/LLL23

Topic: H.02. Human Cognition and Behavior

Support: Marie Curie Fellowship 659680

Title: Making inter-temporal choices in a 3D environment: proximity to rewarding cues weakens self control

Authors: *D. O'CONNOR, R. JANET, J.-C. DREHER;
CNRS, Bron, France

Abstract: Although it has been theorized that physical proximity to reward can lead to more impulsive choices in humans, there has been no systematic study to corroborate this. We developed an immersive inter-temporal choice task, using the Oculus Rift virtual reality headset, to investigate whether mere proximity to immediate appetitive (sweet) rewarding cues increases impulsive choices towards them. We found significant increase in choices for smaller, immediate rewards over larger delayed rewards when they were presented within subjects' grasp (Near; <30cm) compared to when they were presented outside this area (Far; 300cm). Moreover, we observed a correlation between near/far choice ratios and scores on the Barratt Impulsivity Scale (BIS). Specifically, those subjects who reported lower rates of BIS impulsivity were more vulnerable to this proximity choice bias. From an evolutionary perspective, we interpret this tendency for subjects to make more impulsive choices for proximal rewards as an adaptive bias. However, when viewed within the context of a modern consumer society, such a bias could be viewed as potentially detrimental.

Disclosures: D. O'Connor: None. **R. Janet:** None. **J. Dreher:** None.

Poster

748. Computational Models of Reward and Decision Making

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 748.26/LLL24

Topic: H.02. Human Cognition and Behavior

Support: Wellcome Trust

Title: Hierarchical environment representations for efficient planning.

Authors: *D. MCNAMEE, D. WOLPERT, M. LENGYEL;
Computat. and Biol. Learning Lab., Univ. of Cambridge, Cambridge, United Kingdom

Abstract: The production of rational sequences of decisions in complex environments is a hallmark of adaptive intelligence. However, planning is plagued by the curse of dimensionality. To overcome this, it has long been suggested that the nervous system hierarchically partitions or "chunks" states and actions into distinct "modules" thus enabling more efficient and flexible planning in high-dimensional environments. In such a modular representation, planning can first operate at a global level across modules acquiring a high-level "rough picture" of the trajectory to the goal and, subsequently, locally within each module in order to "fill in the details". However, the neural and computational principles which underpin such representations remain obscure. In order to address this, we developed a normative theory of hierarchical environment decomposition optimized for a given planning algorithm based on optimally trading off the average (information theoretic) description length of path planning at the global and local levels. We used the resulting modular representations to identify behavioral and neural signatures of efficient state-space representation during sequential decision-making.
Our model provides a unifying account for a diverse range of hitherto unrelated phenomena at multiple levels of representation and behaviour across mammalian species. For example, recent neuroimaging work (Javadi et al., BNA, 2014) has identified human hippocampal activity which parametrically covaries with changes in a state's ``degree centrality'' (a topological measure of connectedness) during virtual spatial navigation in London's Soho. We show that this unexplained neural correlation could be due to the initiation of local search processes based on an optimal decomposition of the Soho environment. In (Bonasia et al., Hippocampus, 2016), it was verified that the mental navigation of goal-directed trajectories exhibits the phenomenon of compressed replay. Our model predicts the power-law scaling of such route compression as a function of path length they observed. Furthermore, our model suggests that the emergence of start/stop signals in prefrontal cortex (Fujii & Graybiel, Science, 2003) and striatum (Jin & Costa, Nature, 2010), and "task-bracketing" (Smith & Graybiel, Neuron, 2013) is due to environment representations which are efficiently coded for the production of goal-directed

behavioural sequences and normatively justifies a variety of statistical and representational characteristics experimentally observed in these neural populations.

Disclosures: **D. McNamee:** None. **D. Wolpert:** None. **M. Lengyel:** None.

Poster

748. Computational Models of Reward and Decision Making

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 748.27/LLL25

Topic: H.02. Human Cognition and Behavior

Title: Computational analyses on the generation process of self-directive responses in neural systems controlled by internal chaotic fluctuations

Authors: ***M. HIRABAYASHI**, H. OHASHI;
Ohashi Toriumi Lab., Dept. of Systems Innovation, Sch. of Engin., The Univ. of Tokyo, Tokyo, Japan

Abstract: Objective
Although recent remarkable advances in brain science, the neural coding mechanisms of higher brain functions, such as perception, memory, learning and thinking, are less understood. Here, focusing on the features of a cell system of a neuron as a component of complex adaptive systems that consist of neural networks, we present computational analyses on the possibilities that a neuron can implement such advanced functions through the process of evolution and development.
Methods
We introduce the action of chaotically behaving neurons to Hodgkin-Huxley model which describes the time evolution of the transmembrane potential in terms of membrane currents. Using a spontaneous-firing neuron, properties of response to input signals are investigated. Previous findings indicate that sensory inputs do not induce an action potential but lock the inner state of a spontaneous-firing system by phase modulation [1-2]. In this case, it can be thought that a cell system of spontaneous-firing neuron is in the critical state from a probabilistic transition state of phase and frequency to a locked state of them. Because this kind of critical states is stable to small perturbation and low noise, spontaneous firing is effective to realize robust information processing.
Results
We observed switching phenomena between excitation and inhibition utilizing resonance caused by chaotic fluctuations. Our results show that there are possibilities that internal states of neurons can be controlled through such fluctuations generated on a cell membrane independently of input

signals. This finding means that systems can generate different outputs from the network without any change of the network structure. For example, by decreasing the sensitivity of cells through the control of chaotic fluctuations, input signals can be cut not to be transmitted downstream. On the other hand, insensible signals can be transmitted by increasing the sensitivity. Because there are several candidates that can regulate such fluctuations by internal signals, these processes may play important roles in the emergence of higher brain functions such as thinking or decision making.
Conclusions
We focused on the aspect of complex adaptive nature of a neuronal cell system as an information processing organization and demonstrated that a neuron has the structure to realize self-directive and flexible responses. By revealing the emerging process of advanced functions step by step, the progressive breakthroughs will be brought in the related fields such as brain science and artificial intelligence.
1. T. Kenet et al., Nature, vol. 425, pp. 954-956, 2003.
2. J. Fiser et al., Nature, vol. 431, pp. 573-578, 2004.

Disclosures: **M. Hirabayashi:** None. **H. Ohashi:** None.

Poster

749. Decision Making and Reasoning

Location: Halls B-H

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Topic: H.02. Human Cognition and Behavior

Support: Knut and Alice Wallenberg Foundation

Stanford Center for Cognitive and Neurobiological Imaging

NSF IGERT

Title: Distributed representation of task context by fine-scaled subnetworks in prefrontal cortex

Authors: ***M. L. WASKOM**, A. D. WAGNER;
Dept. of Psychology, Stanford Univ., Stanford, CA

Abstract: The cerebral cortex exhibits functional organization at multiple spatial scales. While there is increasing understanding of how the cortex is organized into large-scale networks, which are themselves composed of areas, less is known about the principles that govern finer scales of functional organization. To address this question, we measured task-evoked responses and spontaneous activity from human prefrontal cortex using high-resolution fMRI. Subjects

performed a context-dependent perceptual decision-making task in which they followed a shifting rule to discriminate the features of a bivalent visual stimulus. Multivariate decoding analyses indicated that frontoparietal control network regions in the inferior frontal sulcus (IFS) contained distributed representations of the task context (the rule). We sought to understand the organization of the information underlying these results. Using single-voxel estimates of context preference, we identified populations of voxels with reliable differences in activation for decisions made under the two contexts. These voxels were distributed throughout the IFS, but they also exhibited significant spatial clustering that could not be accounted for by the inherent smoothness of the data. We then tested whether voxels with similar context preferences were organized into subnetworks by analyzing spontaneous correlations in both task residuals and resting-state data. These analyses revealed that spontaneous correlations between voxels with similar context preferences were approximately twice as large as those between voxels with opposing context preferences. Although spatial proximity was a major influence on spontaneous correlation strength, increased correlations between voxels with similar preferences could not be fully accounted for by measures of proximity. These results suggest that distributed representations in human association cortex are supported by fine-scaled subnetworks, which illustrates a novel perspective on the functional organization of higher-order cognitive processes.

Disclosures: **M.L. Waskom:** None. **A.D. Wagner:** None.

Poster

749. Decision Making and Reasoning

Location: Halls B-H

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Program#/Poster#: 749.02/LLL27

Topic: H.02. Human Cognition and Behavior

Support: NRF-2015R1D1A1A01058093

Title: Even-related potentials correlated with the evaluation of service-to-service brand extension

Authors: ***T. YANG**, E. SEOMOON, S.-P. KIM;
Dept. of Human Factors Engin., Ulsan Natl. Inst. of Sci. and Technol., Ulsan, Korea, Republic of

Abstract: Brand extension refers to the introduction of new service or goods to the market by exploiting an established brand. Brand extension can reduce advertising costs and market entry barrier, whereas it can also jeopardize brand equity by weakening a positive association with the original brand. Therefore, understanding how consumers evaluate brand extension is important to development of brain extension strategy. Many studies have investigated neural mechanisms

underlying consumer's evaluation of brand extension. Yet, the previous studies have focused only on goods-to-goods brand extension to date. Since it is more difficult to categorize services rather than goods, the same neural mechanism may or may not be involved in service-to-service brand extension. In the present study, we investigated whether neural process for evaluating service-to-service is similar to that for goods-to-goods brand extension via an event-related potential (ERP) analysis. We used a prime-probe paradigm in which participants were given a series of stimuli. A popular service brand name in one of the four service categories (e.g., airline, hotel, internet shopping mall service, and bank) was presented as a prime stimulus (S1), followed by one of the seven extension service names (e.g., online travel information service, travel agency service, legal counseling service, etc.) as a probe stimulus (S2). Participants were asked to evaluate the suggested brand extension without explicit response. After the experiment, Participants assessed each S1-S2 combination using the 7-point Likert scale (e.g., similarity between the service of S1 brand and S2 service). Based on the survey result, every S1-S2 combination was divided into three groups: high-, moderate- and low- similarity groups. EEG signals were measured during the experiment. The ERP analysis revealed the modulation of N400 components over the right frontal region with the S1-S2 similarity. The N400 amplitude was significantly increased in the order of moderate, high, and low similarity ($p < 0.05$). However, we did not observe P2 at frontal areas that was shown in the previous studies on goods-to-goods brand extension evaluation. Our results indicate that consumers may use a different cognitive process during evaluating service-to-service brand extension compared to goods-to-goods brand extension. To the best of our knowledge, this is the first study to explore neural activity associated with service-to-service brand extension.

Disclosures: **T. Yang:** None. **E. Seomoon:** None. **S. Kim:** None.

Poster

749. Decision Making and Reasoning

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 749.03/LLL28

Topic: H.02. Human Cognition and Behavior

Title: Oxytocinergic modulation of human adaptive communication and broadband neuronal dynamics

Authors: ***A. STOLK**[1], I. KOKAL[2], M. DE BOER[2], R. OOSTENVELD[2], I. TONI[2];
[1]Knight Lab., Helen Wills Neurosci. Institute, UC Berkeley, Berkeley, CA; [2]Donders Inst., Nijmegen, Netherlands

Abstract: Oxytocin is a neuromodulator thought to influence human social and affiliative behavior. Here we explore a neurophysiological mechanism through which oxytocin might influence how we adjust our communication to others. Recent evidence suggests oxytocin might reduce social anxiety and promote sensitivity to social cues. Yet, to date, the neurophysiological mechanism by which oxytocin supports human adaptive social behavior remains largely unknown. This study addresses this issue by exploring the neurocognitive consequences of oxytocin administration during human adaptive communication. Fifty-eight males participated in a randomized, double-blind, placebo-controlled experiment involving the intranasal administration of oxytocin (24 IU). The participants were asked to communicate non-verbally with two addressees, an adult or a child, in an experimentally controlled interactive setting. In reality, a confederate blindly performed the role of both adult and child addressee, such that the two addressees were matched in their level of understanding and differed only in terms of the communicator's prior beliefs. We used magnetoencephalography (MEG) to capture the neuronal dynamics evoked during the live communicative interaction and contrasted neuronal activity evoked after oxytocin administration with that of placebo controls. We focus on broadband changes in neuronal activity, building on recent work showing the importance of those broadband shifts, i.e. changes in mean firing rates of neuronal populations, for integrating driving afferences with contextual information [1]. Oxytocin tonically up-regulated broadband neuronal activity in a right-lateralized fronto-temporal circuit previously found to support human adaptive non-verbal communication in a state-dependent manner. Communicators with stronger broadband fronto-temporal activity adjusted more readily their signals to what the addressees actually understood during the interaction, and were less biased by their prior beliefs about the abilities of those addressees. These findings point to a fundamental neuronal mechanism through which oxytocin influences how we adapt our social behavior, in line with previous work linking tonic up-regulation of broadband activity with on-line communicative alignment between interlocutors [2]. [1] Stolk, Verhagen, Schoffelen, Oostenveld, Blokpoel, Hagoort, van Rooij, Toni. Neural Mechanisms of Communicative Innovation, *Proc Natl Acad Sci U S A 2013* [2] Stolk, Verhagen, Toni. Conceptual Alignment: How Brains Achieve Mutual Understanding, *Trends Cogn Sci 2016*

Disclosures: A. Stolk: None. **I. Kokal:** None. **M. de Boer:** None. **R. Oostenveld:** None. **I. Toni:** None.

Poster

749. Decision Making and Reasoning

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Topic: H.02. Human Cognition and Behavior

Support: NRF Grant 2015-R1A2A2A04006136

Title: Voxel-wise mapping of the cingulate cortex in impression formation

Authors: *J. AHN[1], Y. NAH[2], S. HAN[1,2];
[1]Dept. of Cognitive Sci., [2]Dept. of Psychology, Yonsei Univ., Seoul, Korea, Republic of

Abstract: People vary in processing information regarding social others. We conducted an fMRI study to investigate the voxel-wise neural mechanisms on forming impressions while incongruent social information about a person are provided. Participants were shown a male face with four characteristics adjectives consecutively presented beneath. Two of adjectives explained him positively whereas the other two gave negative information. To investigate the order and anchoring effects of incongruent information, two positive adjectives were followed by two negative adjectives in half of the trials and vice versa in the other half. Then participants evaluated impression toward the person on eight-point scale. The bilateral cingulate cortex were selected as a priori regions of interest (ROIs) and were functionally parcellated into anterior (ACC) and posterior cingulate cortex (PCC). Amygdala was also identified as an ROI. Each adjective was considered as single trial and beta estimates of every voxel in ROIs were extracted. We established two ideal models using step functions one of which hypothesized that beta estimates would gradually decrease while four adjectives were presented (model 1) suggesting that impression would be anchored in the initial information. The other model hypothesized the opposite neural activity pattern (model 2) which assumed that neural activities would increase to resolve the conflicts of information and the latter information would alter general impressions toward the person. Parameters that minimized sum of squared deviations between ideal models and signals from each voxel were estimated with GRG nonlinear algorithm of Solver add-in in Microsoft Excel and mapped in the cortex. The results showed that voxels of PCC were mainly fit to model 1 significantly better than model 2 suggesting impression anchoring to initial information. We also found functionally parcellated dorsal ACC from ventral ACC in that voxels in the dorsal ACC followed model 1 while those in the ventral ACC showed similar patterns with model 2. In addition, voxels of the amygdala showed inconsistent patterns of fitting value while generally following model 1 except that small clusters in the left ventral amygdala were fit to model 2. These data-driven findings demonstrate that activity patterns of each brain region during impression formation can be represented in the cingulate cortex via voxel-wise model estimation; the PCC yields anchoring effects of first impression regardless of valence of information and the ACC is involved with resolution of information incongruity.

Disclosures: **J. Ahn:** None. **Y. Nah:** None. **S. Han:** None.

Poster

749. Decision Making and Reasoning

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Topic: H.02. Human Cognition and Behavior

Support: CIHR grant MOP-115197

NSERC grant 327317-11

Title: Neural dynamics surrounding the individual arising of spontaneous thoughts

Authors: ***M. GIRN**[1], M. ELLAMIL[2], L. WARD[1], K. CHRISTOFF[1];
[1]Univ. of British Columbia, Vancouver, BC, Canada; [2]Max Planck Res. Group Neuroanatomy and Connectivity, Munich, Germany

Abstract: Spontaneous thinking constitutes a ubiquitous aspect of our mental life and has increasingly become a hot topic of research in cognitive neuroscience. To date, functional neuroimaging studies of spontaneous thought have revealed general brain recruitment centered on a combination of default mode network and executive regions. The precise temporal relationship between the regions recruited, however, has yet to be fully elucidated. A previous study by our group employed fMRI to this end (Ellamil et al., Neuroimage, 2016), and characterized regional recruitment in blocks of 2 seconds prior to, during, and after the spontaneous arising of thought. A primary finding was the recruitment of medial temporal lobe (MTL) structures 2 seconds prior to thought onset, suggesting a specific role of the MTL in the inception of spontaneous thoughts. The limited temporal resolution of fMRI, however, strongly constrains the precision of such analyses. The present study seeks to validate and extend our previous findings by employing EEG to perform a more fine-grained analysis of the temporal dynamics of brain activity underlying spontaneously arising thoughts. To do this, we performed independent component analysis (ICA) on high-density EEG recorded using the same paradigm. Pilot data has recovered multiple sources of electrical brain activity in line with those previously identified by our fMRI study. Preliminary results suggest a temporal evolution of recruitment that originates in limbic structures and spreads to cortical heteromodal regions. This temporal evolution of spontaneous thought osberved at the neural level may reflect the cognitive evolution of spontaneous thought as it moves from initial generation, to affective appraisal, to subsequent cognitive elaboration.

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Poster

749. Decision Making and Reasoning

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Topic: H.02. Human Cognition and Behavior

Support: Research grant from Precourt Energy Efficiency Center to NS, AS, and BK

Research grant from Center for Cognitive and Neurobiological Imaging to NS and BK

Title: The influence of eco-labeling on neural predictors of energy-efficient purchases

Authors: *N. SAWE, A. SAHOO, G. TANG, B. KNUTSON;
Stanford Univ., Stanford, CA

Abstract: Consumers routinely underinvest in energy efficiency by purchasing appliances with high long-term energy costs. The Energy Star labeling program was implemented to highlight energy-efficient products and has been largely successful in increasing investment in energy efficiency, yet consumers still vary widely in their valuations of both the Energy Star label and energy consumption data. In a functional magnetic resonance imaging (fMRI) task involving the incentive-compatible purchase of CFL light bulbs, we aimed to understand neural mechanisms that promote purchases of energy-efficient goods, as well as individual differences in choices. Specifically, we focused on individual differences in temporal discounting, which theorists have posited may contribute to underinvestment in energy efficiency.

During the fMRI task, subjects (n=36) chose in 64 trials whether to buy CFL bulbs which varied with respect to the presence or absence of an Energy Star label, annual energy cost, and price. One trial was randomly chosen at the end of the experiment to count as a binding decision; if purchased, the bulb's cost was deducted from the endowment and provided to the subject. Subjects also completed a questionnaire to ascertain their discount rates.

Logistic regression analyses using volume of interest FMRI activity to predict purchases revealed that choices to purchase CFL light bulbs were positively predicted by activity in the nucleus accumbens ($Z=3.43$, $p<0.001$) and caudate ($Z=3.62$, $p<0.001$) and negatively predicted by activity in the MPFC ($Z=-2.25$, $p=0.025$). Further, the presence of the Energy Star label significantly increased activity in the nucleus accumbens ($T=2.97$, $p=0.003$) and caudate ($T=3.38$, $p<0.001$) during the decision phase.

With respect to individual differences, those with higher discount rates showed more activation in response to the Energy Star label in the anterior insula, caudate, and nucleus accumbens. Logistic regressions revealed that in individuals with high discount rates, purchases were positively predicted by nucleus accumbens activity ($Z=2.95$, $p<0.01$) and negatively predicted by anterior insula activity ($Z=-2.15$, $p<0.05$), but in individuals with low discount rates, purchases were negatively predicted by MPFC activity ($Z=-3.16$, $p<0.01$). These individual differences are

consistent with the notion that people with high discount rates may rely more on affective processes during purchasing, and so may respond more prominently to eco-labeling. These findings may help policy makers to refine eco-labeling to further increase investment in energy efficiency and optimize behavioral interventions across a diverse range of consumers.

Disclosures: **N. Sawe:** None. **A. Sahoo:** None. **G. Tang:** None. **B. Knutson:** None.

Poster

749. Decision Making and Reasoning

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 749.07/LLL32

Topic: H.02. Human Cognition and Behavior

Title: Forming a desired belief: a computational account

Authors: ***D. CAHILL**, T. SHAROT;
Psychology, Univ. Col. London, London, United Kingdom

Abstract: How do we form beliefs about hidden states? Past work suggests that such inferences are guided not only by the available evidence but also by the desirability of that evidence. However, the dynamics of this process are largely unknown. Here we use an accumulator model—the drift diffusion model—to describe the formation of such beliefs and the role of valence in this process. In a series of experiments we ask participants to play a slot machine which can either be in a desirable state (more likely to produce gains than losses) or undesirable state (more likely to produce losses than gains), and require them to report which state they believe the machine to be in as they play. Markedly, participants were more likely to report the slot machine as being in a desirable state than an undesirable state than the objective evidence warranted, and were faster to reach desirable conclusions, despite being incentivized for accuracy. Applying a drift diffusion model to this data revealed (1) a larger drift rate towards desirable beliefs than undesirable beliefs and (2) a response bias towards desirable beliefs. This suggests humans both accumulate desirable evidence faster and have a predisposition to hold desirable beliefs. The results offer a computational account of the role of valence in belief formation.

Disclosures: **D. Cahill:** None. **T. Sharot:** None.

Poster

749. Decision Making and Reasoning

Location: Halls B-H

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Program#/Poster#: 749.08/LLL33

Topic: H.02. Human Cognition and Behavior

Support: ANR-BALAV1

ANR-BASCO

France-Israel Laboratory of Neuroscience

France-Israel High Council for Science and Technology

Title: Is there "value" in preference?

Authors: ***D. HANSEL**[1], L. LEBOVICH[2], Y. LAVI[3], R. DARSHAN[2], Y. LOEWENSTEIN[2]; [1]CNRS, Paris, France; [2]The Edmond and Lily Safra Ctr. for Brain Sciences, The Hebrew Univ. of Jerusalem, Jerusalem, Israel; [3]The Inst. of Life Sciences, The Hebrew Univ. of Jerusalem, Jerusalem, Israel

Abstract: It is generally believed that choices between alternative options are based on the subjective value associated with these alternatives. In this framework, choice preference in favor of one alternative over the other reflects a difference in subjective values. Here we challenge this framework and show theoretically and experimentally how preference emerges without value. Theoretically, we studied choice preference in decision-making networks by considering the dynamics of a standard decision-making network model composed of two large populations of spiking neurons that compete via lateral inhibition. We demonstrate using numerical and analytical methods that substantial response bias naturally emerges from the *microscopic* dynamics of the network. This happens even in symmetric settings, in which the two competing populations are statistically identical and receive identical input. We show that the reason for this bias is that the degree of spatial heterogeneities in the network architecture (quenched disorder) and stochasticity of neuronal activity (fast noise) identically scale with the size of the network. As a result, we predict that the *distribution* of response biases would be wide, even in a symmetric decision-making task.Experimentally, we studied choice behavior of human participants in two experiments, in which the "values" associated with the two alternative actions are equal. In the first experiment, we measured the response bias of participants in a symmetrical version of the bisection task and found that the distribution of response biases across the population of participants is substantially and significantly wider than expected by chance, as predicted by our theory. In the second task, participants repeatedly chose the order of two sequentially executed motor plans and we measured their order preference. Again, we found that

despite the task being temporally symmetric, the distribution of order-preference across the population of participants was wide. We conclude that consistent choice preference, even in symmetrical settings, is a natural consequence of the dynamics of competing neural networks and does not require any additional assumptions about a valuation system.

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Poster

749. Decision Making and Reasoning

Location: Halls B-H

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Program#/Poster#: 749.09/LLL34

Topic: H.02. Human Cognition and Behavior

Support: Science of Learning Research Centre

Title: Frontoparietal and salience network activity precedes voluntary decisions in a virtual environment

Authors: *N. RENS[1], H. BURIANOVÁ[2], S. BODE[3], R. CUNNINGTON[1];
[1]Queensland Brain Inst., [2]Ctr. for Advanced Imaging, The Univ. of Queensland, Brisbane, Australia; [3]Melbourne Sch. of Psychological Sci., The Univ. of Melbourne, Melbourne, Australia

Abstract: Studies have demonstrated that freely made decisions are associated with activation of regions of the frontoparietal network (Rowe et al., 2005, Thimm et al., 2012) but it is unclear to which part of the decision-making process this activity relates. We examined whether freely made choices differed from instructed choices in the period of choice selection, prior to both motor preparation and response execution. We further examined to what extent activity at this time would predict the type of decision being made. We created a novel decision-making paradigm in a virtual environment that 22 participants (15 female, mean age 23) performed during fMRI. Participants were required to search for rewards by selecting one of three differently coloured doors at the end of a virtual corridor. The Instructed condition cued participants to choose a specific door colour. The Free condition required participants to choose a colour door of their own volition; however, participants were not permitted to make the same choice as in the previous trial. This rule was implemented to hinder the influence of patterns of choice history on the current decision. The time leading up to the decision point was divided into two epochs, with the doors becoming visible only during the second half. This permitted a time-window of analysis that isolated the process of decision generation, prior to the planning of specific motor responses.

We firstly used multi-voxel pattern analysis (MVPA) with a whole-brain searchlight approach (Kriegeskorte et al., 2006) to investigate where the patterns of neural activity differed between Instructed and Free choice conditions. We found that decision type could be decoded, with a minimum decoding accuracy of 70%, in widespread regions of the brain, predominantly in the frontal and parietal lobes. In order to examine the networks in more detail, we then conducted a mean-centred Partial Least Squares (PLS) analysis (McIntosh et al., 1996). This revealed an increase in activity for Free over Instructed choices that included areas in two key networks: the frontoparietal network and the salience network with bilateral insula. These networks are thought to be involved in attentional processing and the integration of relevant stimuli in order to allow evaluation between options. In conclusion, these findings suggest that activity of the frontoparietal network may be involved in the choice selection process of intentional decision-making and that this occurs at a conceptual level, independent of action preparation.

Disclosures: **N. Rens:** None. **H. Burianová:** None. **S. Bode:** None. **R. Cunnington:** None.

Poster

749. Decision Making and Reasoning

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 749.10/LLL35

Topic: H.02. Human Cognition and Behavior

Support: Duke Institute for Brain Sciences Incubator Award

Title: The temporal parietal junction tracks evidence of guilt in mock criminal scenarios

Authors: R. M. CARTER[1], S. HAKIMI[1], J. M. PARELMAN[1], J. R. LAW[2], N. VIDMAR[2], J. A. G. SKENE[2], D. H. BESKIND[2], J. M. PEARSON[2], *J. SKENE[3];
[1]Univ. of Colorado, Boulder, Boulder, CO; [3]Dept. of Neurobio., [2]Duke Univ., Durham, NC

Abstract: The United States criminal justice system is imperfect, as evidenced by recent high-profile exonerations reminding us that errors in the criminal justice system can and do occur. Reducing the frequency of these errors requires an understanding of the processes by which jurors arrive at a decision of guilt. Of fundamental concern are the juror's goals when making this decision. In addition to the trial evidence, is the process of deciding guilt based on measuring threat, maximizing personal utility, weighing moral culpability, or a combination of each of these? In order to better understand the nature of the neural processes underlying the assessment of guilt, we performed fMRI scanning on 32 jury eligible participants while details of mock criminal cases were revealed to them in a staged fashion. Information provided to the jurors included the type of crime, physical evidence, prior criminal history, and the presence of a

witness. Each of these pieces of evidence contributed to decisions of guilt. With each revealed piece of information, the accumulation of evidence in each scenario was modeled as a sum of the weight of each factor, with model weights determined by previous large-scale behavioral studies. Whole-brain corrected univariate analyses indicated that a single, bilateral region of the brain, the temporal-parietal junction (TPJ) correlates with the linear accumulation of evidence of guilt. Using the perceptual decision making literature as a parallel, evidence accumulation within only the TPJ distinguishes it from decisions about deserved punishment, which have been shown to incorporate assessments of harm or personal utility, associated with activations in the amygdala and striatum. Activation in the TPJ could then imply a dependence on measuring theory of mind which could include moral responsibility, intent, or interpreting another's actions.

Disclosures: R.M. Carter: None. **S. Hakimi:** None. **J.M. Parelman:** None. **J.R. Law:** None. **N. Vidmar:** None. **J.A.G. Skene:** None. **D.H. Beskind:** None. **J.M. Pearson:** None. **J. Skene:** None.

Poster

749. Decision Making and Reasoning

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 749.11/LLL36

Topic: H.02. Human Cognition and Behavior

Title: Spontaneous blink rate correlates with financial risk taking

Authors: *R. C. WILSON[1], E. SHERMAN[2];
[1]Univ. of Arizona, Tucson, AZ; [2]BASIS Scottsdale, Phoenix, AZ

Abstract: Dopamine has long been thought to play a role in risky decision-making, with higher baseline levels of dopamine thought to drive risk seeking behavior. Despite this progress, the exact nature of the relationship between dopamine and risk taking is incompletely understood. For one thing, dopamine has different effects on different receptors, which are themselves distributed differently in different areas of the brain. Moreover, some studies have found that dopamine genes and drugs have different effects depending on range of other factors including gender, sensation seeking and whether the risks involve gains or losses.
In this work we sought to shed more light on dopamine's role in risk taking by using a remarkable relationship between the rate at which someone blinks and the amount of D2-related dopamine in striatum (Karson, 1983; Elsworth et al., 1991). We therefore hypothesized that if blink rate reflects dopamine and dopamine drives risk taking, then we should see a positive relationship between individual differences in blink rate and risk taking across the population. To investigate the relationship between blink rate and risk seeking, we measured blink rates and

financial risk taking in 45 participants ranging in age from 18 to 59. Blink rates were measured by manually counting blinks in videos of the participants "staring into space". Financial risk taking was measured using a survey in which each question offered participants a choice between a certain outcome (e.g. 100% chance of $240) and a risky outcome (e.g. 25% chance of $1000). The survey consisted of nine questions and included questions with both gain and loss framing of the outcomes.
Consistent with previous work linking dopamine to risky decisions, we found a strong positive correlation between blink rate and the number of risky choices a participant made (Spearman's $\rho(43) = 0.57$, $p = 4.45 \times 10e\text{-}5$). Contrary to prior findings, we found that this correlation was not dependent on age or gender and was identical for both gain and loss framing.
These findings suggests that D2-related dopamine plays a crucial and quite general role in determining financial risk taking across the population. In addition our work demonstrates the potential of spontaneous blink rate as a simple method of probing dopamine for decision-making research.
Karson, C.N. (1983). Brain 106 (3), 643-653
Elsworth, J.D. et al. (1991). J. Pharmacol. Exp. Ther. 259, 595-600.

Disclosures: R.C. Wilson: None. **E. Sherman:** None.

Poster

749. Decision Making and Reasoning

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Topic: H.02. Human Cognition and Behavior

Support: Marie Skłodowska-Curie Fellowship

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Bertarelli foundation

Title: Metacognition across senses and combination of senses

Authors: *N. FAIVRE[1,2], E. FILEVICH[4], G. SOLOVEY[5], S. KUHN[4], O. BLANKE[3];
[1]CNRS, Paris, France; [2]Lnco, [3]EPFL, Genève, Switzerland; [4]MPI, Berlin, Germany; [5]FCEyN, Buenos Aires, Argentina

Abstract: Humans have the capacity to access and report their own mental states. A widely used method to study these metacognitive capacities is to have observers do a challenging perceptual task followed by a confidence judgment regarding task performance. In this operationalization,

metacognitive accuracy can be quantified as the correspondence between the subjective confidence judgments and objective task performance. While more evidence has been gathered regarding the mechanisms and brain areas involved in metacognitive monitoring, some of the core properties of metacognition remain largely unknown. One of the central questions is whether, and to what extent, metacognitive monitoring should be considered domain-general: is the computation of confidence fully independent of the nature of the task (i.e., domain-generality), or does it also involve task-specific components (i.e., domain-specificity)? Here we sought to further describe the commonalities and specificities of metacognition across vision and audition, and extend it to touch, a sensory modality that has been neglected so far. We first examined correlations between metacognitive accuracy during a visual, auditory, and tactile discrimination task (Experiment 1). Next, extending our paradigm to conditions of audiovisual stimulation, we quantified for the first time the links between unimodal and multimodal metacognition, and assessed through computational modeling how multimodal confidence estimates are built (Experiment 2). Finally, we investigated the neural mechanisms of unimodal and multimodal metacognition by replicating Experiment 2 along with electroencephalographic recordings combined with amplitude and frequency analyses (Experiment 3). Together, these results provide new insights regarding the behavioral, neural, and computational bases of metacognition across different senses, and, crucially, when they are combined. Based on behavioral and neural evidence, we propose that domain-generality during metacognitive judgments are driven by decisional factors during the first-order task which are modality-independent (e.g., reaction times), and thus likely candidates to explain partial domain-generality in metacognition.

Disclosures: **N. Faivre:** None. **E. Filevich:** None. **G. Solovey:** None. **S. Kuhn:** None. **O. Blanke:** None.

Poster

749. Decision Making and Reasoning

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Topic: H.02. Human Cognition and Behavior

Support: Grant-in-Aid for Scientific Research 15K12055, from the Japan Society for the Promotion of Science

Staff research grant from Hyogo College of Medicine

Title: Natural respiratory types on cognitive flexibility and recognition during human discrimination task

Authors: *N. H. NAKAMURA[1], M. FUKUNAGA[2], Y. OKU[1];
[1]Dept. Physiology, Hyogo Col. of Med., Nishinomiya, Japan; [2]Natl. Inst. of Physiological Sci., Okazaki, Japan

Abstract: A coordination with timing and patterns of breathing is thought to be crucial for making an action and succeeding a task performance during sports, such as football (soccer), baseball, tennis, and martial arts. Indeed, autonomic functions including respiration can be controlled by human prefrontal cortex, which is primarily involved in executive functions and cognitive flexibility, such as set shifting and behavioral inhibition. To understand the respiratory patterns regarding cognitive flexibility, we investigated changes in respiration and vagal activity during a complex discrimination task, which demands high cognitive flexibility. We found at least four natural breathing types in 38 healthy subjects during the discrimination task. These breathing types were composed of mainly three elements (periodicity, extension of apnea/expiration, and randomness): 1) periodic type (P, 60.5%, n=23), 2) periodic + apnea/expiration-extending type (PE, 15.8%, n=6), 3) random type, in which the respiration swings quickly between inspiration and expiration or makes two/three peaks during expiration (R, 18.4%, n=7), and 4) apnea/expiration super-extending type (5.3%, n=2). Then, we further analyzed respiration and heart beats during the task in subjects with P, PE, and R respiratory patterns. We showed that the study and test phases in the task had shorter respiratory cycle, smaller respiratory amplitude, and smaller respiratory sinus arrhythmia (RSA) amplitude as compared to those during the pre-task state. Interestingly, the test phase, which demands set shifting, behavioral inhibition, and recognition, had shorter expiratory phase and larger numbers of heart beats in inspiratory phase as compared to those during the pre-task state and study phase. These results suggest that respiratory patterns are well coordinated by cognitive flexibility and/or recognition. More importantly, we found that subjects with R patterns had smaller RSA amplitudes as compared to those with P or PE subjects, suggesting that the sympathetic tone is more likely to be activated in R-type subjects. We will discuss about a further possible correspondence of respiratory types to autonomic patterns regarding a cognitive performance.

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Poster

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Topic: H.02. Human Cognition and Behavior

Support: CIHR grant MOP-115197

NSERC grant 327317-11

Title: Why does the mind move? Explaining the dynamics of mind-wandering through experience-sampling

Authors: *A. HERRERA-BENNETT[1], Q. RAFFAELLI[2], K. CHRISTOFF[2];
[1]Psychology, [2]Univ. of British Columbia, Vancouver, BC, Canada

Abstract: The bulk of the mind-wandering literature has primarily operationalized spontaneous thought as a function of being on- versus off-task, with rates of stimulus-independent or task-unrelated thoughts accounting for 30 -50% of daily life (e.g., Killingsworth & Gilbert, 2010). This task-centric perspective has predominately shaped the way in which researchers have attempted to capture spontaneous mental activity, and has in turn neglected the dynamic property of mind-wandering, namely the tendency of the mind to move in a relatively unconstrained fashion within a broad conceptual scope of ideas from one moment to the next (e.g., Christoff et al., in press). The present study capitalized on experience-sampling methodology to developed novel probe items that aimed at empirically measuring thoughts' dynamic qualities outside of the lab, where thoughts are expected to be subject to a host of potentially concurrent and ongoing tasks and at the mercy of contextual influences. Participants responded to thought probes at randomized times over the course of multiple days, being asked to rate their thoughts on a number of different dimensions (7-point Likert scales), including the apparent randomness and speed in the movement of their current thoughts, their thoughts' relatedness to the current activity (*on- vs. off-task*), as well as the extent to which they felt drawn to think about something (*automatic constraints*). In line with prior research, subjects reported being off-task approximately a third of the time, and experienced similar frequencies of thoughts that were characterized by moderate to high levels of randomness. Our results suggest an overall significantly negative relationship between these two dimensions: The more participants reported being off-task, the more their thoughts moved freely or randomly. This relationship, however, differed considerably among subjects, suggesting that the pervading dynamic quality of mind-wandering is not wholly captured by definitions of mind-wandering exclusively centered around task-focus. This suggests a need to identify what influences the free movement of thought above and beyond those constraints posed by task-focus.

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Poster

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Max Planck UCL Centre for Computational Psychiatry and Ageing Research

Wellcome Trust 091593/Z/10/Z

Gatsby Charitable Foundation

Title: Fast sequences of non-spatial state representations in humans

Authors: *Z. KURTH-NELSON[1], M. ECONOMIDES[2], R. DOLAN[1], P. DAYAN[3];
[1]Max Planck UCL Ctr. for Computat. Psychiatry, [2]Wellcome Trust Ctr. for Neuroimaging, [3]Gatsby Computat. Neurosci. Unit, Univ. Col. London, London, United Kingdom

Abstract: Fast internally generated sequences of neural representations are suggested to support learning and online planning. However, these sequences have only been studied in the context of spatial tasks, and never in humans. Here, we recorded magnetoencephalography (MEG) while human subjects performed a novel non-spatial reasoning task. The task required selecting paths through a set of six visual objects. We trained pattern classifiers on the MEG activity elicited by direct presentation of the visual objects alone, and tested these classifiers on activity recorded during periods when no object was presented. During these object-free periods, the brain spontaneously visited representations of approximately four objects in fast sequences lasting on the order of 120 milliseconds. These sequences followed backward trajectories along the permissible paths in the task. Thus, spontaneous fast sequential representation of states can be measured non-invasively in humans, and these sequences may be a fundamental feature of neural computation across tasks.

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Poster

749. Decision Making and Reasoning

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Topic: H.02. Human Cognition and Behavior

Support: NSC 102-2410-H-002-004-MY3

Title: P3 like late positivity dissociates young and older adult value-based decision processing

Authors: *P. CHEN[1], H.-Y. HUNG[1,2], J. GOH[1,3,4];
[1]Grad. Inst. of Brain and Mind Sci., Col. of Medicine, Natl. Taiwan Univ., Taipei, Taiwan; [2]Dept. of Physics, [3]Dept. of Psychology, [4]Neurobiological and Cognitive Sci. Ctr., Natl. Taiwan Univ., Taipei, Taiwan

Abstract: Value-based decision-making requires the accurate assessment of the likelihoods of outcomes. We previously found that age influences frontostriatal neural activity involved in such valuative processes in older adults. To evaluate temporal counterparts of these age effects on frontostriatal valuative processing, we measured young and older adult event-related potential (ERP) responses towards different likelihoods of reward and cost, 11 young (mean age (SD) = 23 (1.9) yrs; 6/5 males/females) and 11 older (mean age (SD) = 68 (4) yrs; 3/8 males/females) normal participants performed the lottery choice task (LCT) as continuous EEG (32 channels) activities were recorded. In each trial, participants saw reward (or cost) magnitude (high, middle, or low) and probability (high, middle, or low) information, and either accepted or rejected the stakes. Across reward magnitude levels, older adults showed riskier behavior than young adults, having significantly higher acceptance rates (old = 13.3%; young = 4.2%; $p < .05$) for low probability stakes. We also found a general P3-like late positivity response (450-750ms) across the three probability conditions from anterior-to-posterior sites (Fz, Cz, Pz) and focused on this component. Overall, older adults showed an enhanced late positivity amplitude for both high and low probability conditions (mean (SD) amplitude; high, 6.3 uV (1.0); middle, 4.7 uV (1.1), and low, 5.5 uV (1.1)) whereas young adults showed an enhanced amplitude only for the high probability condition (mean (SD) amplitude; high, 6.4 uV (1.0); middle, 5.7 uV (1.0); and low 5.5 uV (1.1)) (F= 2.97, p =.06). Moreover, in middle probability and accepted high probability trials, reward magnitude did not modulate late positivities in young or older adults. In rejected low probability trials, however, lower reward magnitudes significantly decreased the late positivity in old (mean (SD) amplitude; high, 5.1 uV (1.4); middle, 5.7 uV (1.0); and low, 3.2 uV (1.1)) but not young adults (mean (SD) amplitude; high, 3.3 uV (1.4); middle, 2.6 uV (1.0); and low, 3.5 uV (1.1)) (F= 8.03, $p < .05$) . In sum, we identify the P3-like late positivity response as a key ERP indicator of age differences in valuative processing with implications for the temporal dynamic of frontostriatal activity. These results suggest that older adult risk-taking tendency is related to greater salience of low prospects despite costs.

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Poster

749. Decision Making and Reasoning

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Topic: H.02. Human Cognition and Behavior

Support: EPSRC student grant

Title: Assessing selectivity in the basal ganglia: the "gearbox" hypothesis.

Authors: *Z. FOUNTAS, M. SHANAHAN;
Imperial Col. London, London, United Kingdom

Abstract: Low-frequency oscillatory activity has been the target of extensive research both in cortical structures and in the basal ganglia (BG), due to numerous reports of associations with brain disorders and the normal functioning of the brain. Additionally, a plethora of evidence and theoretical work indicates that the BG might be the locus where conflicts between prospective actions are being resolved. Whereas a number of computational models of the BG investigate these phenomena, these models tend to focus on intrinsic oscillatory mechanisms, neglecting evidence that points to the cortex as the origin of this oscillatory behaviour. In this study, we employed a new spiking neural model of the BG circuitry to investigate the relationship of wave properties of entrained cortical inputs, dopamine and the transient effectiveness of the BG, when viewed as an action selection device. We found that cortical frequency, phase, dopamine and the examined time scale, all have a very important impact on the ability of our model to select. Our simulations resulted in a canonical profile of selectivity, which we termed selectivity portraits. Taking together, our results suggest that the cortex is the structure that determines whether action selection will be performed and what strategy will be utilized while the role of the BG is to perform this selection. Some frequency ranges promote the exploitation of actions of whom the outcome is known, others promote the exploration of new actions with high uncertainty while the remaining frequencies simply deactivate selection. Based on this behaviour, we propose a metaphor according to which, the basal ganglia can be viewed as the "gearbox" of the cortex. Coalitions of rhythmic cortical areas are able to switch between a repertoire of available BG modes which, in turn, change the course of information flow back to and within the cortex. In the same context, dopamine can be likened to the "control pedals" of action selection that either stop or initiate a decision. Finally, the frequency of active cortical areas that project to the BG acts as a gear lever, that instead of controlling the type and direction of thrust that the throttle provides to an automobile, it dictates the extent to which dopamine can trigger a decision, as well as what type of decision this would be. Finally, we identified a selection cycle with a period of around 200 ms, which was used to assess the biological plausibility of the most popular architectures in cognitive science. Our results agree well with experimental observations, provide new justifications and insights into oscillatory phenomena related to decision making and reaffirm the role of the BG as the selection centre of the brain.

Disclosures: **Z. Fountas:** None. **M. Shanahan:** A. Employment/Salary (full or part-time): Imperial College London.

Poster

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Topic: H.02. Human Cognition and Behavior

Support: NIH AG017586

NIH AG038490

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NIH AG052943

Title: Connnectome analysis of category naming fluency in frontotemporal degeneration

Authors: *M. GROSSMAN[1], P. COOK[2], C. MCMILLAN[3], C. JESTER[4], K. RASCOVSKY[4], A. HALPIN[4], R. LANGEY[4], O. KOFMAN[4], J. GEE[5];
[1]Dept Neurol., Howard Hughes Med. Inst. - Univ. of Pennsylvania, Philadelphia, PA; [2]Dept Radiology, Univ. of Pennsylvania, Philadelphia, PA; [3]Neurol., Univ. of Pennsylvania, PHILADELPHIA, PA; [4]neurology, [5]radiology, university of Pennsylvania, pHILADELPHIA, PA

Abstract: A data-driven eigenanatomy approach previously showed that a combination of grey matter (GM) and white matter (WM) was more robust statistically than either of these modalities alone in defining the neuroanatomic basis for the common neuropsychological task - letter-guided category naming fluency (FAS) - in patients with frontotemporal degeneration (FTD) (Cook et al, 2015). Recent advances in imaging analysis have adopted a game theory approach to evaluate the combined role of GM and WM in the identification of the anatomic associations of neurodegenerative syndromes such as Alzheimer's disease and FTD. Here, we used game theory analyses to evaluate the large-scale network subserving FAS performance in non-aphasic patients with behavioral variant FTD (bvFTD). We examined 46 patients meeting research criteria for bvFTD (Rascovsky et al, 2011) and 46 healthy, demographically-matched controls. Patients performed a letter-guided category naming fluency task (FAS). All participants also obtained T1-weighted MRI with 1mm isotropic voxels, and cortical GM was partitioned into 98 regions using the OASIS label set. Diffusion-weighted imaging used a 30-direction protocol. All WM voxels were seeded, and deterministic tractography was used to identify streamlines between 2 different GM regions (nodes). Fractional anisotropy (FA) was averaged across streamlines to weight projections (edges) between nodes. We obtained several measures of network integration and segregation. Our results showed widespread reduction in FA projections between nodes in frontal and left temporal regions. Regression analysis related FAS performance

to these projections ($p<0.05$, Bonferroni-corrected). In addition, we found reduced degree centrality (number of projections between each node and other nodes in the network) and reduced closeness centrality (shortest projections between each node and all other network nodes) in frontal-temporal regions in bvFTD ($p<0.05$, FDR-corrected), and these measures of integration were related to FAS performance in frontal-temporal regions. While there were reduced frontal-temporal hubs (nodes through which shortest-path projections pass, or betweenness centrality) in bvFTD, this was not related to FAS performance. We also found reduced cluster-efficiency (a measure of segregation related to modularity) in frontal-temporal regions of bvFTD, but this was not associated with FAS performance. We conclude that large-scale neural networks are compromised in bvFTD, and that the deficit in these patients is most closely related to a specific disorder of network integration that reflects poorly integrated frontal-temporal nodes.

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Poster

749. Decision Making and Reasoning

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NSF CRCNS BCS 130934

Title: Neural change-detection without the need to detect changes: leaky integration approaches Bayesian optimality

Authors: C. K. RYALI[1], *A. J. YU[2];
[1]Univ. of California San Diego, La Jolla, CA; [2]Cognitive Sci., Univ. of California San Diego, LA Jolla, CA

Abstract: In recent years, there has been much progress in understanding how humans and animals learn about statistical regularities in the environment to make optimal decisions, as well as how they track changes sudden changes in environmental statistics based on noisy data. Broadly speaking, this problem has been studied using two kinds of data, those that are continuous or ordinal-valued, and those that are binary or categorical. The first kind has often been modeled using variants of the Kalman filter, while the latter kind has been found to be

successfully captured by the Dynamic Belief Model (Yu & Cohen, 2009), or DBM, a variant of a Bayesian hidden Markov model that assumes the observations to be iid distributed from a hidden variable, which itself goes occasional and discrete changes. We previously showed that DBM can explain a variety of behavioral phenomena: sequential adjustment effects in 2AFC discrimination tasks, inhibitory control (e.g. stop-signal) tasks, and explicit prediction tasks, as well as providing a normative explanation for matching-type behavior in a multi-choice visual search task and exploration stochasticity in multi-arm bandit tasks. However, the computational and representational complexity of DBM make it unlikely to be exactly implemented in the brain. Here, we show that DBM can be very well approximated by a simple linear exponential filter, i.e. linearly combining previous observations using weights that decay exponentially into the past. We derive the parametric form of this approximation, showing that the discount factor is a linear function of the assumed hazard rate for change in the corresponding DBM, and that the predictive prior probability of each possible outcome can be computed solely as a function of empirical statistics related to this outcome, with no need to interact with data related to the other alternatives. Interestingly, this approximation is exactly equivalent to the dynamics of an appropriately tuned leaky integrating neuron. Our results also imply that Bayesian learning is achieved to very good approximation by a fixed learning rate in the linear exponential filter, or equivalently a fixed gain in the leaky integrating equation, with no need to explicitly compute the probability of a change having taken place; To conclude, this work makes interesting predictions for how statistical learning and choice behavior depend on experimental variables such as the number of outcome alternatives; it also has direct implications for identifying the neural substrate of statistical learning and change-detection based on noisy categorical data.

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Poster

749. Decision Making and Reasoning

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Topic: H.02. Human Cognition and Behavior

Title: Θband occurrence pattern at the onset of a voluntary movement in humans

Authors: *S. KAWASHIMA[1], A. MORI[1], N. MINAKAWA[1], Y. KITA[2];
[1]Grad. Sch. of Literature and Social Sci., Nihon Univ., Tokyo, Japan; [2]Keisen Univ., Faculty of Human and Social Studies, Japan

Abstract: It is known in theta band (3-7 Hz) of electroencephalogram (EEG) studies, theta band in front midline area is associated with focused attention. This band is called frontal midline

theta rhythm (Fmθ), frequency is 6-7 Hz. However, the theta band used in these studies had a wide range (3-7Hz), it is difficult to determine whether Fmθ (6–7Hz) was involved in the results. Therefore, the present study examined to classify theta band into low theta band (3-5Hz; Lθ) or high theta band (6-7Hz; Hθ), and use a 128-channel digital electroencephalograph to compare their appearance patterns in athletes and non-athletes at the onset of voluntary movement.
The subjects were 18 healthy right-handed men, 9 of which were athletes (kendo practitioners; 18-21 years of age (20.6 ± 1.1)) and 9 were non-athletes (18–22 years of age (19.5 ± 2.1 years)). The experimental task consisted of a voluntary movement task, which was pushing a button. The task was performed in sets of 30 and repeated 3 times, for a total of 90 times; a 3-min break time was provided between trials.
The EEGs were recorded on the scalp by 128 channels. The standard electrode during the recording was positioned on the vertex of the head and the grounding electrode was on the Fpz (frontal Midline-Parietal). Recording conditions were defined as follows: 500 Hz as sampling frequency, 0.1 Hz as low frequency filter, 200 Hz as high frequency filter and 100 kΩ or lower as resistance value between the electrode and the scalp.
As a results, the occurrence pattern of the Lθ was especially localized in the prefrontal area from an onset of a voluntary movement. On the other hand, the occurrence pattern of the Hθ was localized in the frontal midline area from 2 seconds before the button press, was localized in the prefrontal area from an onset of a voluntary movement. In addition, there potential powers ware significantly higher in the athletes.
The present result suggested that there is a difference between the occurrence pattern of the Lθ and Hθ at the onset of a voluntary movement. In addition, the potential power of the θband high in athletes, exercise was suggested may enhance the focused attention.

Disclosures: **S. Kawashima:** None. **A. Mori:** None. **N. Minakawa:** None. **Y. Kita:** None.

Poster

749. Decision Making and Reasoning

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 749.21/LLL46

Topic: H.02. Human Cognition and Behavior

Support: Internal University Grant

Title: The use of mobile EEG in a pilot study focused on the impact of low current brain stimulation on math understanding and calculations.

Authors: ***R. A. HOUSER**[1], D. FONSECA[2], E. O'CONNOR[3], W. WEBBER[3], I. HEIM[2], A. IRWIN[3];

[1]Educational Studies, The Univ. of Alabama, Tuscaloosa, AL; [2]Engin., [3]Educational Studies, Univ. of Alabama, Tuscaloosa, AL

Abstract: *Introduction*
This project was designed to identify neural correlates of math calculations central to successful functioning in STEM programs of study. The intent was to determine the impact of the low brain current stimulation in effort to enhance performance on math calculations. Outcome data were collected through EEG data collection and performance in math calculations.
Participants
Participants were undergraduates registered for a pre-calculus course at a research university in the southeast. The pilot study included 10 participants, 7 females and 3 males. The age of participants ranged from 19 to 22. Participants were paid $100 for completing six sessions.
Procedures
We initially collected baseline data of brain activity, five minute EEG recording. We used a mobile EEG amplifier so we could move from the laboratory to the natural environment, the classroom. Participants were randomly assigned to either a control (sham) condition or an experimental condition. The sham condition participants received a 1 mA 30 second administration of transcranial direction current stimulation, tDSC. The experimental condition participants received a 2 mA administration for 20 minutes prior to exposure of the instructional video, math calculation performance and classroom experience. Placement for anodal stimulation for both groups was at the P3 electrode based on the 10-20 International System. P3 and the left intraparietal sulcus which has been associated with math calculations. The cathode electrode was place over T4 based on the 10-20 International System and is associated with fine motor and should not impact math performance. Participants were initially shown a video demonstration of pre-calculus problems. Following the video participants completed a calculation based on the video. EEG data were collected while the participants completed the calculations. Participants completed six sessions, a baseline and five sessions involving exposure to math calculations videos and completion of math calculations.
Results
Spectral data were analyzed using ASA Lab software (ANT Neuro). Initially we removed artifacts from raw EEG data. The artifact filter was set at 75 μHz and -75 μHz. Once artifacts were removed data were converted to average spectral outcomes based on four second intervals. Four second intervals were randomly selected from each EEG data collection session. We will discuss preliminary pilot data comparing those receiving tDCS prior to viewing pre-calculus problems and while completing problems.

Disclosures: **R.A. Houser:** None. **D. Fonseca:** None. **E. O'Connor:** None. **W. Webber:** None. **I. Heim:** None. **A. Irwin:** None.

Poster

750. Human Cognition

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 750.01/LLL47

Topic: G.07. Other Psychiatric Disorders

Title: Negative valence and dysmodulation of orbitofrontal cortical activity in borderline personality disorder: Sensitivity of top-down attention to facial valence

Authors: *R. MANIMALETHU[1], K. RAMASESHAN[2], A. BURGESS[2], P. SOLOFF[3], V. DIWADKAR[2];
[1]Wayne State Univ., Brighton, MI; [2]Wayne State Univ., Detroit, MI; [3]Univ. of Pittsburgh, Pittsburgh, MI

Abstract: Background Attention and stimulus valence independently strongly modulate fMRI-estimated cortico-limbic activity. Generally, fMRI responses represent a combination of top-down (attention & regulatory) and bottom-up (stimulus-driven) processes (Logothetis, 2009), particularly in the context of emotional processing. Inducing congruence between top-down processes ("what is being looked for"?) and bottom-up processes ("does the stimulus conform to what is being looked for"?) may be a way to enhance the sensitivity of the brain's emotion regulatory systems such as the orbitofrontal (OFC) and cingulate cortices (ACC) , and may be particularly valuable in characterizing clinical syndromes characterized by emotional dysregulation. Here we compared effects of positive (+ Context & + Stimuli) and negative (- Context & - Stimuli) congruence in healthy controls (HC) and patients with Borderline Personality Disorder (BPD), an Axis II personality disorder characterized by emotional dysregulation and hyper-lability (Leichsenring et al, 2011). **Methods** fMRI was collected (3T, Siemens Verio) while 42 BPD subjects and 29 healthy controls performed a modified CPT task (Soloff et al, 2015). A block of trials began with instructions specifying the context (+ve or -ve) indicating the affective class of faces on which to enhance vigilance. During the block, participants were shown a series of faces (1 Hz, jittered ISI) with letters ("A" or "X") imposed on the bridge of the nose, where "X" was a target only if facial valence was consistent with the context. Event related analyses (SPM 8) were used to estimate fMRI responses to congruent events in each class (+ve and -ve). Each participant contributed these two contrast maps to a second level using random effects analyses of variance, with Group (HC vs. BPD) modeled as independent factor and valence (-ve vs. +ve) modeled as non-independent factor. The results are specifically focused on Group x Valence interactions ($p<.05$). **Results** In HC, modulation of OFC activity increased from positive to negative congruence, an effect notable given the OFC's cardinal role in emotion regulation under conditions of -ve valence (Underwood et al., 2016). By comparison, in BPD, the modulation of the OFC decreased from positive to negative congruence suggesting reduced modulation under conditions of high emotion regulatory demand.

Conclusions The results imply a "turning off" in BPD of emotion regulatory regions under conditions when negative stimuli are evaluated in negative context. Manipulating top-down and bottom-up mechanisms of evaluating valence may enhance the search for mechanisms of emotional dysregulation in syndromes such as BPD.

Disclosures: R. Manimalethu: None. **K. Ramaseshan:** None. **A. Burgess:** None. **P. Soloff:** None. **V. Diwadkar:** None.

Poster

750. Human Cognition

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 750.02/LLL48

Topic: H.02. Human Cognition and Behavior

Title: Individual differences in frontal alpha asymmetry and achievement goals mastery versus asymmetry

Authors: *M. E. MINEAR, W. SCOTT, 82071, M. COWEN, M. BROWN, T. HOLMES, D. MECHAM, W. MILLER, M. RADICH, A. STILL;
Psychology, Univ. of Wyoming, Laramie, WY

Abstract: Relative differences in left v. right frontal lobe activity at rest as measured by inverse alpha power (8-13Hz) have been shown to correspond to individual differences in approach v. avoidance tendencies as measured by self-report instruments such Carver & White's BIS/BAS Scale with BAS approach motivation associated with greater left resting activity and BIS associated with greater right activity. BAS corresponds to a behavioral tendency to seek out positive and negative reinforcement while BIS reflects sensitivity to punishment & reward and behavioral inhibition. Achievement goals are the reasons why an individual pursues a goal. Two dimensions of achievement goals have been proposed, mastery v. performance and approach v. avoidance motivation.
We hypothesized that frontal asymmetry would correspond to the approach- avoidance dimension such that both mastery and performance avoidance goals would be related to right sided activity while approach goals would correlate with greater left activity.
Methods
23 participants (14 female) completed both the BIS/BAS Personality Survey (Carver & White, 1994) and the Achievement Goal Questionnaire-Revised (AGQ-R; Elliot & Murayama, 2008). To measure frontal asymmetry, we used the resting paradigm reported in many previous studies of temperament and affect. EEG data were collected from the participants while they quietly rested in a darkened room for 8 one minute EEG trials, 4 eyes-open, 4 closed. Data were

collected using a high density 128 channel system (EGI) with a 250Hz sampling rate and a bandpass 0.1 to 100Hz filter. Log transformed power data in the alpha range for channels corresponding to frontal electrodes F3, F5, and F7 on the left and F4, F6 and F8 on the right were computed using EEGLab. Asymmetries scores were computed by averaging the 3 channels on the left and the right and subtracting left activity from right.
Results
BIS scores were negatively correlated with asymmetry scores (r = -.42) i.e. higher BIS scores were associated with greater right frontal activity as has been reported previously. BIS was positively correlated with all four achievement goals. However, only performance goals (both approach and avoidance) showed a relationship with frontal asymmetry scores similar to BIS scores i.e. associated with greater right frontal activity while neither mastery goal was associated with asymmetry.
Our hypothesis was not supported. Instead, these data suggest that performance goals in which a person evaluates her performance against others is related to right hemisphere activity regardless of approach or avoidance motivation.

Disclosures: **M.E. Minear:** None. **W. Scott:** None. **M. Cowen:** None. **M. Brown:** None. **T. Holmes:** None. **D. Mecham:** None. **W. Miller:** None. **M. Radich:** None. **A. Still:** None.

Poster

750. Human Cognition

Location: Halls B-H

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Program#/Poster#: 750.03/LLL49

Topic: G.07. Other Psychiatric Disorders

Support: FRQS, Fonds de la recherche Québec Santé

Title: D-lysergicdiethylamide (LSD) modulates dopaminergic neurons of Ventral Tegmental Area invivo through a pleiotropic mechanism involving D_2 and $TAAR_1$receptors

Authors: D. DE GREGORIO[1], L. POSA[1], R. OCHOA-SANCHEZ[1], R. MCLAUGHLIN[1,2], S. COMAI[1,3], *G. GOBBI[1];
[1]McGill Univ., Montreal, QC, Canada; [2]Washington State Univ., Pullman, WA; [3]Vita-Salute-San Raffaele Univ., Milano, Italy

Abstract: Background: D-lysergic diethylamide (LSD) is a hallucinogenic drug with potent psychotropic effects. LSD may produce psychotic-like symptoms such as visual, tactile, acoustic hallucinations, change in body perception, and synesthesia. Traditionally, the psychotropic properties of LSD have been attributed to its effects at the level of the serotonin (5-HT) system,

but given the role of dopamine (DA) in the pathogenesis of psychosis, an interaction of LSD with the DA system cannot be excluded. Current knowledge regarding a possible *in-vivo* interaction of LSD with mesolimbic dopamine (DA) neurons of the ventral tegmental area (VTA), the main source of DA innervation in the brain, is limited. *In-vitro* studies have demonstrated that LSD has affinity for DA receptors, in particular D2 receptors, and for trace-amine associated receptor 1 ($TAAR_1$), in addition to its affinity for 5-HT_{1A} and 5-HT_{2A} receptors. Thus, we examined the *in-vivo* effects of cumulative doses of LSD on VTA DA neurons, and attempted to identify the underlying neurobiological mechanisms. **Methods**: Using *in-vivo* electrophysiology, we first studied the effects of cumulative doses of LSD (5-120 ug/kg, i.v.) on VTA DA neurons and on 5-HT neurons in Sprague-Dawley male adult rats. Secondly, we tested the contribution of 5-HT_{1A}, D_2, and $TAAR_1$ receptors in the mechanism of action of LSD upon VTA DA neurons by using the D_2 antagonist haloperidol (HALO,50 µg/kg, i.v.), the 5-HT_{1A} antagonist WAY-100,635 (WAY,500 µg/kg, i.v.), or the novel synthesized trace amine-associate receptor 1 ($TAAR_1$) antagonist EPPTB (5 mg/kg, i.v.). **Results**: LSD dose-dependently decreased VTA DA firing activity at doses higher (30-120 µg/kg, i.v.) than those (5-20 µg/kg, i.v.) inhibiting dorsal raphe 5-HT neurons (ED50: 71.8 vs. 13.1 µg/kg; $F(2,7)=39.34$, $P<0.0001$). The inhibitory effects of LSD on VTA DA firing activity were prevented by HALO, WAY and EPPTB, suggesting the involvement of D2, 5-HT1A and $TAAR_1$ receptors in the mechanism of action of LSD. Interestingly, the single i.v. injection of 5 mg/kg EPPTB increased VTA DA firing activity compared to vehicle (2.89±0.60 vs 2.15±0.34 Hz, $P=0.002$). **Discussion**: These results demonstrate that LSD at low doses affects the 5-HT system while at high doses strongly modulates DA mesolimbic neuronal activity with a pleiotropic mechanism of action involving 5-$HT_{1A,}$ D_2 and $TAAR_1$ receptors. Moreover, for the first time, we demonstrated that EPPTB exerts a tonic enhancement of DA firing activity *in vivo*.

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Poster

750. Human Cognition

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Program#/Poster#: 750.04/LLL50

Topic: H.02. Human Cognition and Behavior

Support: CIHR grant MOP-115197

NSERC grant 327317-11

Title: The wandering brain: Individual differences in grey and white matter structure predict frequency of goal-related and emotionally positive self-generated thoughts

Authors: *S. SHETH[1], K. C. R. FOX[2], M. S. JARRETT[2], M. GIRN[2], M. DIXON[2], A. RAUSCHER[2], K. CHRISTOFF[2];
[1]Psychology, [2]Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Self-generated thought has been most famously tied to activity in brain regions of the default mode network (DMN), although a number of regions outside the DMN appear to be consistently involved as well (Fox et al., 2015). Little is know, however, about how the brain's anatomical structure might vary in individuals with differing overall patterns of thought. For instance, individuals show marked differences in the frequency with which their thoughts are goal-related (vs. unrelated) and emotionally positive (vs. neutral or negative). Are these individual patterns of thinking related to individual differences in neuroanatomy? We sought to explore the relationships between these individual propensities and both grey matter concentration (using high-resolution T1 anatomical MRI scans) and white matter integrity (using diffusion tensor imaging). Following a morphometric neuroimaging scan, we allowed subjects to rest and think freely, interrupting their thinking at random intervals with occasional thought probes. A total of 120 probes asked subjects (i) whether their thoughts arose spontaneously, or whether they were intentionally directing them; (ii) whether thoughts were related to their current concerns and goals in life, or not; and (iii) whether they were emotionally pleasant, unpleasant or neutral. Overall individual difference scores were calculated for each participant (e.g., proportion of positive thoughts), and correlated with whole-brain grey matter concentration and tract-based white matter integrity. We identified distinctive patterns of both grey and white matter structure that were correlated with individual propensity toward spontaneously arising vs. intentionally directed thoughts; thoughts related vs. unrelated to current concerns and goals; and emotionally pleasant vs. unpleasant thoughts. Moreover, these differences were observed in many regions beyond the DMN. Our results suggest that distinctive individual tendencies in the content and valence of self-generated thinking are linked to correspondingly distinctive neuroanatomy. Our results also show that the brain structure of people who tend to have negative, non-goal-related thoughts is quantitatively distinct from those who tend to spontaneously produce positive, goal-related thinking, suggesting possible implications for understanding clinically relevant alterations of spontaneous thought such as depressive rumination.

Disclosures: **S. Sheth:** None. **K.C.R. Fox:** None. **M.S. Jarrett:** None. **M. Girn:** None. **M. Dixon:** None. **A. Rauscher:** None. **K. Christoff:** None.

Poster

750. Human Cognition

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Topic: G.07. Other Psychiatric Disorders

Support: NIDA Grant 1R01DA026932

NIDA Grant 1R21DA029464

NIDA Grant 1R21DA027149

NIMH Grant 1R21MH086880

Title: Neural abnormalities underlying feedback processing in psychopathy: Processing deficits remain with attention controlled

Authors: *M. S. SHANE[1,2], B. DARLING[1];
[1]Univ. of Ontario Inst. of Technol., Oshawa, ON, Canada; [2]The Mind Res. Network, Albuquerque, NM

Abstract: Real-world and experimental evidence converge in demonstrating that psychopathic individuals exhibit marked deficits learning to adapt maladaptive behaviors. These deficits have been theorized to result from reduced attention to relevant performance feedback, however the precise nature of these attentional abnormalities have yet to be established. The present study utilized functional magnetic resonance imaging (fMRI) to investigate the neural underpinnings of feedback-related learning deficits in psychopathy. 72 antisocial participants recruited through probation parole received a PCL-R interview, and completed a forced-choice learning task while in the scanner. In line with reduced attention to feedback in psychopaths, results indicated that psychopathic individuals (n = 15) showed reduced bilateral caudate response compared to nonpsychopathic individuals (n = 25) following both positive and negative feedback. However, when the duration of attention paid to feedback was controlled by including it as a parametric modulator, neural reductions *extended further,* into left dorsal anterior cingulate cortex (dACC), bilateral insula, left caudate and orbitofrontal cortex (OFC). Thus, even with the duration of attention to feedback controlled, psychopathic individuals showed reduced signaling in response to performance feedback. These results suggest that feedback-related learning deficits in psychopathy may extend beyond current attentional hypotheses, and may include additional underlying disruption in the utilization of attended-to feedback. Further characterization of these processing abnormalities may have important implications for understanding and managing the learning-related abnormalities in psychopathy.

Disclosures: **M.S. Shane:** None. **B. Darling:** None.

Poster

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Topic: H.02. Human Cognition and Behavior

Support: CTSI Grant TL1TR001428

CTSI Grant UL1TR001427

NIH Grant R21AG044862

Title: Neural substrate of fatigue in older adults

Authors: ***S. E. BURKE**[1], I. B. H. SAMUEL[1], Q. ZHOU[1], S. N. SIEGEL[1], J. CAGLE[1], B. M. KLUGER[2], M. DING[1];
[1]Univ. of Florida, Gainesville, FL; [2]Univ. of Colorado Sch. of Med., Aurora, CO

Abstract: Fatigue is an extremely debilitating condition in older adults. It is associated with increased mortality and decreased quality of life. Despite the significant negative impact of fatigue, we know very little about its actual cause. Currently, there are no objective measures to diagnose the condition, and there is no treatment. Past work has suggested a role of white matter disruption in the pathogenesis of fatigue. To test this hypothesis, we recruited 25 older adults between the ages of 60 to 84 years and assessed their fatigue levels by the fatigue severity scale (FSS). These subjects then underwent diffusion magnetic resonance imaging (MRI). From these diffusion MRI data whole-brain Tract Based Spatial Statistics (TBSS) were obtained and compared between subjects with high levels of fatigue and subjects with low levels of fatigue. Differences between the two groups were detected in the white matter pathways surrounding the substantia nigra and anterior cingulate cortex. To further investigate the mechanism behind these white matter changes, we are analyzing the diffusion MRI data for Free Water, a reliable marker of inflammation that is highly specific to the brain. Through this analysis, we aim to show that neuroinflammation leads to white matter breakdown and that this is a measurable objective marker of fatigue in older adults.

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Poster

750. Human Cognition

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 750.07/LLL53

Topic: H.02. Human Cognition and Behavior

Title: Investigating a novel implicit measurement of self-perception

Authors: *A. TEER, C. V. LEONARD, M. AIREY, N. IRFAN, M. T. TREADWAY; PSYCHOLOGY, EMORY UNIVERSITY, Atlanta, GA

Abstract: Growing evidence suggests that self-perception and self-esteem may be difficult constructs to assess using common self-report measures due to reporter biases. Tasks like the Implicit Association Task (IAT) have helped to address these limitations, but the neural circuitry involved in IAT tasks is unknown. In contrast, the neurobiological mechanisms of fear conditioning and extinction are very well characterized. The current study attempts to take advantage of these well-known circuits to test the feasibility of fear conditioning and extinction to self-related imagery as an objective biomarker of self-esteem.

34 healthy volunteers participated in this study. Participants completed self-report measures including the State Self-Esteem Scale (SSES), and had their photo taken at the beginning of the study. Three photographs served as conditioned stimuli (CS), one of which was the photograph of the participant (CS-Self). The CS-Self and another photograph (CS+) were always paired with the UCS. A third was never paired with the UCS (CS-). The unconditioned stimulus (UCS) was a loud unpleasant noise and pupil dilation served as the unconditioned response (UCR), which is a well-validated metric for autonomic arousal. Following a habituation block, participants completed two blocks of fear acquisition (24 trials each) and then two blocks of extinction. Based on contingency awareness ratings, explicit learning rates were comparable across subjects. All data were analyzed using multi-level mixed-effects GLMs in STATA. As expected, a significant main effect of CS type on pupil area (PA) exists such that CS-Self and CS+ images were associated with larger pupil dilation. Additionally, significant interactions existed between CS-type and the Appearance subscale of the SSES (SSES-A), when predicting PA at a trial-by-trial level. During the first acquisition block, this interaction was significant for CS-Self v. CS+ ($p = .025$). During the first extinction block, this interaction was significant for CS-Self v. CS- ($p= .032$). In both cases, the interaction of CS-type, UCS dislike, and SSES-A was also significant ($p = .037$; $p = .038$).

The aforementioned effects remain significant even when controlling for the ratings of UCS aversiveness. These preliminary findings support the potential for this paradigm to be used as an objective measure of self-esteem.

Disclosures: **A. Teer:** None. **C.V. Leonard:** None. **M. Airey:** None. **N. Irfan:** None. **M.T. Treadway:** None.

Poster

750. Human Cognition

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Program#/Poster#: 750.08/LLL54

Topic: G.07. Other Psychiatric Disorders

Support: NRF-2014M3C7A1062893

Title: Altered prefrontal cortical gray matter volume is associated with the relationship between depression and Internet gaming

Authors: ***J. CHOI**[1], J.-W. CHUN[2], H. CHO[2], J.-Y. KIM[2], K. AHN[3], D. KIM[2];
[2]Dept. of Psychiatry, [3]Dept. of Radiology, [1]Seoul St. Mary's Hosp., Seoul, Korea, Republic of

Abstract: Playing Internet games has become one of the most popular leisure activities for adolescents and young adults. Adaptive use of Internet games is associated with entertainment and improved spatial cognition, whereas previous studies have been frequently reported depression is prevalent in Internet gaming disorder (IGD). However, the neural mechanism underlying the association between depression and Internet gaming remains unclear. The aim of the current study is to investigate the structural brain alterations related to IGD and examine whether the alteration is associated with the relationship between depression and Internet gaming. Twenty-four controls (CON), who were not played Internet games, 25 subjects with Internet gaming control (IGC), and 22 subjects with IGD underwent structural magnetic resonance imaging. All subjects were males in their 2-30s without psychiatric disorder such as major depressive disorder. Using a voxel-based morphometric (VBM) approach, a voxel-wise whole brain analysis identified gray matter difference in the left dorsolateral prefrontal cortex (DLPFC) among three groups. Post-hoc analysis revealed the IGD group exhibited the lowest GMV in this region compared with the CON and IGC groups. Gray matter volume in the left DLPFC was negatively correlated with the amount of lifetime gaming and level of depression in the Internet gaming users (IGC+IGD groups). Furthermore, a mediation analysis revealed that the lifetime usage had indirect influence on the level of depression through the structural alteration in the DLPFC. The VBM results implicate that the brain structural alteration is involved in IGD. Additionally, the result of the current study may provide an intervention strategy for patients with IGD coexisting depression, one of the most common comorbid psychiatric symptoms of IGD.

Disclosures: **J. Choi:** None. **J. Chun:** None. **H. Cho:** None. **J. Kim:** None. **K. Ahn:** None. **D. Kim:** None.

Poster

750. Human Cognition

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 750.09/LLL55

Topic: H.02. Human Cognition and Behavior

Support: NSERC

FRSQ

CFI

Title: Behavioral phenotyping of impulsivity with a dynamic decision-making task

Authors: ***M. CARLAND**[1], G. DEROSIERE[3], D. THURA[2], P. CISEK[2];
[1]Physiologie, [2]Neurosci., Univ. De Montréal, Montreal, QC, Canada; [3]Inst. of Neurosci., Catholic Univ. of Louvain, Brussels, Belgium

Abstract: *Impulsivity* is a multidimensional construct that represents a well-known vulnerability factor for a variety of clinical disorders including addiction, problem gambling, and other forms of compulsive behavior. In the laboratory, impulsivity is typically measured with a variety of behavioral tasks including *go/no-go* paradigms and the Iowa Gambling Task, whereas researchers and practitioners in the clinical domain often rely primarily on self-reported psychometric batteries. One example is the widely-used *UPPS Impulsivity Scale* (Whiteside & Lynam, 2001, *Pers. & Indiv. Diff.*), which partitions impulsivity into five sub-factors including *Positive-* and *Negative Urgency*, *(Lack of) Premeditation, (Lack of) Perseverance*, and *Sensation-Seeking*.
However, recent meta-analyses have revealed that behavioral- and self-report measures of impulsivity account for separate sources of individual variance in impulsivity-related traits, suggesting that these assessment measures are tapping into distinct and non-overlapping dimensions within a broader underlying construct (Cyders & Coskunpinar, 2012, *J. Res. Personality*). This lack of agreement between measurement approaches thus represents an outstanding challenge to the utility of impulsivity as an explanatory construct in conceptual and clinical domains alike.
Here we present a novel dynamic decision-making task that yields a variety of discrete behavioral measures related to individual differences in risk- and reward sensitivity, response

inhibition, and speed-accuracy preferences. We also report initial findings regarding the relationship of these performance measures to various sub-factors of the UPPS impulsivity scale, thereby bridging the gap between behavioral and self-report measures of impulsivity. We conclude that this task offers the possibility to develop more precise cognitive-behavioral phenotypes of impulsivity, as well as to clarify the potential contributions of these phenotypes to a variety of clinical disorders.

Disclosures: **M. Carland:** None. **G. Derosiere:** None. **D. Thura:** None. **P. Cisek:** None.

Poster

750. Human Cognition

Location: Halls B-H

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Program#/Poster#: 750.10/LLL56

Topic: F.02. Behavioral Neuroendocrinology

Support: NCN OPUS 2014/15/B/HS6/03792

Title: Altered orbitofrontal reactivity during reward processing among pathological gamblers and problematic pornography users

Authors: ***M. K. GOLA**[1], M. WORDECHA[2], G. SESCOUSSE[3], M. LEW-STAROWICZ[4], M. WYPYCH[5], B. KOSSOWSKI[5], S. MAKEIG[1], A. MARCHEWKA[5], M. POTENZA[6];
[1]UC San Diego, INC, SCCN, San Diego, CA; [2]Inst. of Psychology, Polish Acad. of Sci., Warsaw, Poland; [3]Radboud University, Donders Inst. for Brain, Cognition and Behavior, Nijmegen, Netherlands; [4]III Dept. of Psychiatry, Inst. of Psychiatry and Neurol., Warsaw, Poland; [5]Lab. of Brain Imaging, Neurobio. Center, Nencki Inst. of Exptl. Biol. of Polish Acad. of Sci., Warsaw, Poland; [6]Departments of Psychiatry and Neurobiology, Child Study Ctr. and CASAColumbia, Yale Sch. of Med., New Haven, CT

Abstract: Pornography consumption has become highly prevalent in part given Internet availability. Approximately 70% of males and 20% of females aged 18-30 years use pornography weekly. For most people, pornography viewing is a form of entertainment, but for some individuals problematic pornography use (PPU) accompanied by excessive masturbation is a reason for treatment seeking (Gola et al., 2016). Such observations raise a number of societally, scientifically and clinically important questions: Can pornography consumption be addictive? What are the neural mechanisms underlying PPU? How might one best conceptualize PPU and intervene most effectively?
Recently using fMRI methodology we examined brain reactivity towards erotic and monetary stimuli, disentangling cue-related ‘wanting’ from reward-related ‘liking’ among 28 heterosexual

males seeking treatment for PPU and 24 matched controls (Gola et al., in press). Compared with control subjects, PPU subjects showed increased activation of brain reward circuits (ventral striatum) specifically for cues predicting erotic pictures but not for cues predicting monetary gains, which exactly mimics results of previous study with the same method on individuals with gambling disorder (Sescousse, et al., 2013)
Here we focused on other brain region involved in reward processing - orbitofrontal cortex (OFC). As it had been shown, evolutionally older posterior OFC in healthy subjects is involved in processing of primary rewards (food and sex), while anterior OFC process secondary rewards (such as money or social reinforces). According to this state of art aOFC is in our study it was the only ROI expressing higher activations for monetary gains than erotic rewards in control subjects. But interestingly, for PPU subjects the aOFC was more active for erotic pictures than monetary rewards, while pOFC remained unchanged. The amount of this shift in aOFC was related to PPU severity measures. Among subjects witch pathological gambling opposite pattern of changes was observed: pOFC was activated more for monetary rewards, while aOFC activations remained unchanged when compared to controls.
Our results suggests that PPU subjects may experience difficulties in differentiating between value of erotic and non-erotic rewards similarly to pathological gamblers in case of monetary and non-monetary rewards. Our results show also that PPU resembles neural and behavioral patterns well-described in gambling disorder. The findings suggest that for some people pornography may be addictive and that behavioral treatments for addictions, warrant investigation in PPU.

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Poster

750. Human Cognition

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Program#/Poster#: 750.11/LLL57

Topic: H.01. Animal Cognition and Behavior

Title: A metacognitive model of personality.

Authors: *K. MOGI;
Sony Comp Sci. Lab., Shinagawa-Ku, Japan

Abstract: In personality research, the big five model (openness, conscientiousness, extraversion, agreeableness, and neuroticism) is considered to provide a standard description of people's traits (Digman 1990, Shrout and Fiske 1995). The variability in personality has been linked to

academic performance (Poropat 2009), work success (Mount and Barrick 1998), and the tendency to innovate (Fairweather 2012).
Although the big five model is based on a large body of research and is statistically robust, one of the drawbacks is the descriptive nature of its methodology. In order to quantitatively characterize personality traits, we need to have a dynamic and quantitatively structured model of personality, which, in turn, might be compared and linked to the traditional personality model. Uncertainty is one of the most important environmental and interaction elements in the makeup of personality. People have different attitudes toward the handling of uncertainty, and risk taking and/or aversion. The human brain's reward systems (e.g. dopamine pathways) are known to modulate their activities based on the (perceived) nature of uncertainties involved (Schultz 1998). In development, secure base (Bowlby 1990) provides the basis for people's exploration of uncertain rewards. From the theoretical perspective, the balance between exploitation and exploration has been shown to be important in reinforcement learning (Sutton and Barto 1998) Here I present a generic model of personality based on a subject's dynamic reaction pattern to actual and perceived uncertainties in the interaction with the environment. The subject's perceived level of secure base, as well as the metacognition of the contexts involved (Redford 2010), are integrated to give a generic agent model compatible with the available data. Elements of emotion connected to this model is described, with other elements of emotion accounted for as secondary traits arising from the processing of information related to the interaction with the environment in the presence of uncertainty.

Disclosures: K. Mogi: None.

Poster

750. Human Cognition

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 750.12/LLL58

Topic: H.02. Human Cognition and Behavior

Title: Age-related differences in the organization of large-scale functional brain networks during successful memory formation

Authors: *G. S. WIG[1,2], F. ALHAZMI[1], M. Y. CHAN[1], N. K. SAVALIA[1];
[1]Ctr. for Vital Longevity, Univ. of Texas at Dallas Ctr. for Vital Longevity, Dallas, TX; [2]Dept. of Psychiatry, Univ. of Texas Southwestern Med. Ctr., Dallas, TX

Abstract: We recently reported age-related differences in the organization of resting-state brain networks (Chan et al., 2014). Increasing age was associated with decreasing segregation of functional sub-networks (systems); lesser segregation was also associated with poorer memory

ability, independent of age. While these results highlight the potential age-related differences in ‘intrinsic’ network organization, they don’t reveal how task-oriented information processing may modify brain network organization, and whether this may differ with age.
Here we examined large-scale network patterns defined by both resting-state functional connectivity (RSFC) and task-evoked functional connectivity in a large group of participants sampled from a wide range of the healthy adult lifespan (The Dallas Lifespan Brain Study; N=208; 20 to 89 yrs). Task-based connectivity was characterized by analyzing participants' blood-oxygen-level dependent (BOLD) activity from an event-related visual discrimination fMRI task; this scanned task was followed by a surprise memory test which allowed us to examine differences in brain connectivity associated with subsequently remembered versus forgotten items. Brain networks were constructed using a pre-defined set of nodes (volumetric regions of interest). Task network edges were calculated between all node pairs using a psychophysiological interaction analysis (PPI). RSFC network edges were calculated between all node pairs using a Fisher-z-transformed Pearson's correlation of their resting-state time-course. Independent of age, system segregation decreased during task performance compared to rest. Further, while younger adults exhibited greater network segregation during encoding of items that were subsequently remembered versus those that were subsequently forgotten, older participants did not exhibit these trial-type segregation differences. Importantly, we also found age-related differences in patterns of task-based connectivity associated with successful memory formation. Younger adults exhibited greater successful encoding-related connectivity between nodes associated with control and default operations (association systems), while older adults exhibited greater successful encoding-related connectivity between nodes of sensory-motor systems and between nodes of association to sensory-motor systems. These results suggest that aging is associated with differences in the organization of large-scale networks both at rest and in the context of goal-directed tasks. They also suggest that patterns of large-scale network connectivity associated with successful encoding differ with age.

Disclosures: **G.S. Wig:** None. **F. Alhazmi:** None. **M.Y. Chan:** None. **N.K. Savalia:** None.

Poster

750. Human Cognition

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 750.13/LLL59

Topic: H.02. Human Cognition and Behavior

Support: UM1 CA167552

Title: Long-term vegetable, fruit, fruit juice intake and subjective cognitive decline in men

Authors: *C. YUAN[1], E. FONDELL[1], A. BHUSHAN[2], A. ASCHERIO[1], F. GRODSTEIN[3], W. WILLETT[1];
[1]Nutr., Harvard T.H. Chan Sch. of Publ. Hlth., Boston, MA; [3]Channing Div. of Network Med., [2]Harvard Med. Sch., Boston, MA

Abstract: Background: Inconsistent findings have been reported on the cognitive effects of fruit, vegetable, and juice consumption, and previous studies have had relatively small sample size and limited period of follow up. This study aimed to investigate the long-term associations of vegetable, fruit, and juice intakes across adulthood with later-life subjective cognitive decline (SCD).
Methods: We conducted a prospective evaluation of vegetable, fruit, juice intakes and SCD among participants from the Health Professionals Follow-up Study. The final study included 26,808 men with a mean age of 51 years at enrollment in 1986, and self-reported SCD in 2008-2012. Cumulative average intakes of vegetable, fruit, and fruit juice were calculated from five repeated food frequency questionnaires collected every four years to 2002. SCD was assessed by a 6-item self-reported questionnaire and the average of the two SCD scores was used to classify participants' cognitive function as "good" (54.4%), "moderate" (38.2%) or "poor" (7.4%).
Results: Intakes of total vegetables, fruits, and fruit juice were significantly inversely associated with the odds of moderate or poor cognitive function after controlling for age, smoking, physical activity, BMI, multivitamin use, disease history of diabetes, high blood pressure, elevated high cholesterol level, cardiovascular disease and depression, alcohol intake and total energy intake. The association became null for fruit intake after further adjusting for intakes of total vegetable, fruit juice, coffee, potatoes, legumes, refined grains, fish and processed red meat. In this model the multivariate odds ratios (95% confidence intervals) for vegetable intake (top vs. bottom quintile) was 0.85 (0.77, 0.95), p-trend=0.003 for moderate SCD and 0.69 (0.57, 0.85), p-trend=0.01 for poor SCD. Similar inverse associations were found for green leafy, and carotenoid-rich vegetables. We also observed inverse associations of non-citrus fruit and berry subgroups with poor SCD. For fruit juice intake, the odd ratios were 0.75 (0.68, 0.82), p-trend<0.001 for moderate SCD and 0.64 (0.54, 0.76), p-trend<0.001 for poor SCD.
Conclusions: Our findings support a potential beneficial role of vegetable and fruit juice consumption to reduce cognitive decline in U.S. men.

Disclosures: C. Yuan: None. **E. Fondell:** None. **A. Bhushan:** None. **A. Ascherio:** None. **F. Grodstein:** None. **W. Willett:** None.

Poster

750. Human Cognition

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Program#/Poster#: 750.14/LLL60

Topic: H.02. Human Cognition and Behavior

Support: NIH grant 5R37AG-006265-25

Title: Functional parcellation of the cerebral cortex across the healthy adult lifespan using resting-state functional connectivity

Authors: *L. HAN[1,2], N. K. SAVALIA[2], M. Y. CHAN[1,2], P. F. AGRES[1,2], G. S. WIG[1,2,3]; [1]Sch. of Behavioral and Brain Sci., The Univ. of Texas At Dallas, Dallas, TX; [2]Ctr. for Vital Longevity, The Univ. of Texas at Dallas, Dallas, TX; [3]Dept. of Psychiatry, Univ. of Texas Southwestern Med. Ctr., Dallas, TX

Abstract: Research using non-invasive methods to parcellate functional brain areas has largely focused on parcellating healthy young adult brains by identifying variations in patterns of evoked-activity, connectivity, or anatomy. However, individuals advanced in age exhibit substantial structural and functional differences relative to younger adults. It is unclear whether a parcellation map derived from young adult brains is entirely suitable for understanding the organization and function of older adults' brains.

A number of recent studies have leveraged patterns of resting-state functional correlations (RSFC) to conduct rapid parcellation of the cerebral cortex and subcortical structures. For example, identifying transitions in patterns of RSFC across the cortical surface has revealed locations of putative areal borders (Cohen et al., 2008). Importantly, RSFC parcellation has been shown to correspond well to parcellation information from other modalities such as task-activity and distinctions in architectonics (Wig et al., 2014). In addition to validating the approach, these observations also suggest that RSFC-based parcellation could be used for characterizing and understanding age-related differences in functional parcellation.

We analyzed data from a large sample of healthy adult participants collected under the Dallas Lifespan Brain Study (DLBS; N=238; 20-89 yrs) for whom RSFC and anatomical information was acquired. Participants were separated into 4 age groups to create age group-specific RSFC-defined parcellation maps across the cortical surface, using modifications of previously published procedures for generating group-based parcellations (Wig et al., 2014; Gordon et al., 2015). The current work focused on describing the age-related variations in parcellation by characterizing differences in parcel position/size and functional connectivity in various locations throughout the cerebral cortex.

Parcellation features were qualitatively similar across age groups: the general topographical organization of parcellated areas did not differ vastly from younger to older age. In addition, seed-based RSFC maps generated from a collection of overlapping parcels across groups exhibited largely similar connectivity patterns. Interestingly, the homogeneity of parcels did exhibit age-related differences, with the most prominent differences occurring between the younger (20-34 yrs) and older adult (65+ yrs) parcellations. Together these results suggest that aging may be accompanied by subtle differences in the parcellation of functional areas, possibly brought on by morphometric changes in anatomy that accompany healthy aging.

Disclosures: L. Han: None. **N.K. Savalia:** None. **M.Y. Chan:** None. **P.F. Agres:** None. **G.S. Wig:** None.

Poster

750. Human Cognition

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 750.15/LLL61

Topic: H.02. Human Cognition and Behavior

Support: NIH-NCCIH T32 AT002688

Title: A cognitive stressor for event-related potential studies: the portland arithmetic stress task

Authors: *R. M. ATCHLEY[1], R. ELLINGSON[2], D. KLEE[2], T. MEMMOTT[2], B. OKEN[2]; [1]Neurol., Oregon Hlth. and Sci. Univ. Dept. of Neurol., Portland, OR; [2]Neurol., Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: Background: There is a need for tasks that better define resilience to stress either as the amplitude or duration of stress-related changes. To this end, we developed a cognitive stressor that can be readily adapted for event-related potential studies. We named the task the Portland Arithmetic Stress Task (PAST) and modeled it on the well-validated Montreal Imaging Stress Task (MIST). The goal of this experiment was to evaluate changes in autonomic stress reactivity of biomarkers elicited by the PAST before incorporating event-related measures. Methods: Sixteen older adults (aged 50-80 years, *M* = 59) with Perceived Stress Scores ≥9 were recruited as part of a randomized clinical trial of mindfulness meditation. Participants first completed a set of standard self-assessment scales of stress, depression and neuroticism. Prior to randomization in the meditation study, the PAST was administered during an in-lab baseline assessment visit. The PAST, as a cognitive stressor, is composed of computerized mental arithmetic problems with an adaptive failure algorithm. It attempts to maintain an error rate of approximately 40% for the duration of the test and includes a social-evaluative threat component and auditory feedback components. The titrated difficulty of the arithmetic problems is performed using five levels based on number of numerals (2-4), number of digits in the numbers (1 or 2), types of operators (+, -, x, and /), presence or absence of fractions, and time window length allowed for responses. The task is approximately 10 minutes long and was repeated twice. To control for normal levels of autonomic arousal during cognitive tasks, participants completed a tones task in which they were instructed to press a button in response to the infrequent (occurrence: 15%), higher pitched tone. Several measures of stress were evaluated: blood pressure (BP), heart rate (HR), heart rate variability (HRV), and respiration rate. BP and HR were taken at time points before, during, and after administration of the PAST. Results: BP increased significantly immediately after the task compared to just prior to the task, with systolic increasing from *M*=131 to 137, p=0.035; and diastolic increasing from *M*=79 to 88, p=.026. HR and HRV were unchanged. Respiration rate increased significantly from 14.5 breaths per minute to 16.3 per minute, p=0.017. The relationships of changes in autonomic measures with self-rated

measures will be discussed.Conclusion: The PAST successfully produced deviations in autonomic nervous system markers consistent with stress-induced changes. Further research is needed to determine any cognitive or other changes occurred as a result of this stress task.

Disclosures: **R.M. Atchley:** None. **R. Ellingson:** None. **D. Klee:** None. **T. Memmott:** None. **B. Oken:** None.

Poster

750. Human Cognition

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 750.16/LLL62

Topic: H.02. Human Cognition and Behavior

Support: Rackham Graduate Student Research Grant, 2015

Title: Smoking effects on attentional control: More than just withdrawal?

Authors: ***J. NICOSIA**, C. LUSTIG;
Psychology, Univ. of Michigan, Ann Arbor, MI

Abstract: Addiction is often linked to poor attentional control, especially strong involuntary attention to and distraction from drug-related cues. On the other hand, it has also been hypothesized that individuals and groups (e.g., patients with schizophrenia or attention deficit disorder) with attentional vulnerabilities use psychoactive drugs, especially nicotine, in attempts at self-medication. We tested smokers and non-smokers in the Continuous Temporal Expectancy Task (CTET; O'Connell et al., 2009) with a video distractor (Berry et al., 2014b). The video CTET allows for simultaneous independent assessment of initial focus/level of performance, the ability to maintain performance over time, and the ability to resist distraction. Using genetic and patient groups, we have previously shown that performance, especially the ability to resist the distractor, is related to cholinergic capacity (Berry et al., 2014a; Kim et al., 2015). Contrary to our initial hypothesis, smokers were not differentially vulnerable to distractor videos with smoking-related content. Instead, smokers had generally higher performance overall. Numerical trends also suggested that smokers had less memory for the distracting video content. Preliminary analyses suggest that those who had smoked recently (within the last 60 minutes) out performed non-recent smokers. Non-recent smokers also reported more subjective distraction from the videos, and had increased memory specifically for those distractor videos with smoking-related content. These results are generally consistent with the idea that cholinergic function supports attentional control, in particular the ability to resist external distraction.

Further, they suggest that nicotine may have pro-cognitive effects on attention that go beyond the amelioration of withdrawal-related deficits.

Disclosures: J. Nicosia: None. **C. Lustig:** None.

Poster

750. Human Cognition

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Program#/Poster#: 750.17/LLL63

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant 5R01MH10332402

War Related Illness and Injury Study Center (WRIISC) Fellowship, Palo Alto VA

Title: Resting fMRI signal predicts evoked connectivity with TMS-fMRI

Authors: *D. OATHES[1], M. GOODKIND[2], G. FONZO[3], G. GLOVER[4], B. PATENAUDE[3], A. ETKIN[3];
[1]Psychiatry, Univ. of Pennsylvania, Philadelphia, PA; [2]New Mexico VA Hlth. Care Syst., Albuquerque, NM; [3]Psychiatry, [4]Radiology, Stanford Univ., Palo Alto, CA

Abstract: Resting fMRI is a widely used method for assessing 'intrinsic' brain processes and connectivity. However, maps of these signals are often distributed and little causal information can be gained by examining correlations in time course or amplitude across the resting brain. In this investigation, we sought to determine a possible correspondence between signal amplitudes in the low frequency band typically measured with resting fMRI and brain responses to TMS recorded with fMRI. We therefore regressed site of stimulation signal amplitudes from a previously acquired resting scan onto TMS evoked connectivity (psychophysiological interaction) seeding the site of stimulation to both the central executive (dorsolateral prefrontal cortex) and the salience network (anterior middle frontal gyrus) and found robust evidence for a positive relationship between resting signal amplitudes and TMS evoked connectivity within each targeted network (cluster FWE corrected). Our results suggest that resting fMRI signal predicts causal information flow to distributed networks from prefrontal cortex.

Disclosures: D. Oathes: None. **M. Goodkind:** None. **G. Fonzo:** None. **G. Glover:** None. **B. Patenaude:** None. **A. Etkin:** None.

Poster

750. Human Cognition

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 750.18/LLL64

Topic: H.02. Human Cognition and Behavior

Title: Self-estimates of cognitive abilities predict performance on the NeuroCognitive Performance Test

Authors: *K. D. BIDDLE[1,2], N. F. NG[2], C. SIMONE[2], G. MORRISON[2];
[1]Dartmouth Col., Hanover, NH; [2]Lumos Labs, Inc., San Francisco, CA

Abstract: To determine if perception of cognitive abilities is a good predictor of NeuroCognitive Performance Test (NCPT) score. To investigate how self-estimates (subjective) and NCPT scores (objective), as well as the subjective-objective relationship, are influenced by individual differences.
An email invitation was sent to Lumosity users (~177k) who were asked to complete a questionnaire via www.surveygizmo.com, followed by the NCPT. The questionnaire collected demographics, self-estimate of cognitive abilities relative to same-aged peers, happiness score (Subjective Happiness Scale), optimism score, and depression severity (Beck Depression Inventory (BDI)). The NCPT is a brief, repeatable, online battery of cognitive assessments that measures cognitive performance in multiple domains. Statistical analysis, including calculation of the correlation (Pearson's r) between self-estimates and NCPT scores and cross-demographic differences, was performed using R.
686 total subjects were analyzed. A correlation of $r=0.37$ ($p<0.001$) between self-estimates and NCPT was observed. 69% reported cognitive abilities as "better than peers," while only 55% scored above average on the age-matched NCPT. The 35-45 year-old group showed the strongest relationship between self-estimates and NCPT ($r=0.46$, $p<0.001$). Education positively correlated with self-estimates and NCPT (education-self-estimate: $r=0.2$, $p<0.001$; education-NCPT: $r=0.1$, $p=0.01$), but there was no significant correlation between education and subjective-objective correlation. There was a positive correlation between both happiness and optimism and self-estimates, but not between these covariates and NCPT (happiness-self-estimate: $r=0.17$, $p<0.001$, optimism-self-estimate: $r=0.18$, $p<0.001$). There was a significant, negative correlation between severity of depression and both self-estimates and NCPT score (BDI-self-estimate: $r=-0.22$, $p<0.001$; BDI-NCPT: $r=-0.1$, $p=0.01$). In a linear model of the effect of self-estimate, happiness, optimism, depression, and education, self-estimate showed the only significant effect on the NCPT (adjusted $R^2=0.14$, $p<0.001$).
Self-estimates are reasonably predictive of normalized NCPT scores, though differences exist across gender, age, and country. There is a stronger relationship between self-estimates and NCPT than education and NCPT. People who score higher on happiness/optimism scales rate

cognitive abilities as better, but do not necessarily perform better/worse on the NCPT. The opposite is true for people who score higher on depression scales, who rate themselves less favorably and perform slightly worse on the NCPT.

Disclosures: K.D. Biddle: None. **N.F. Ng:** A. Employment/Salary (full or part-time): Lumos Labs, Inc. **C. Simone:** A. Employment/Salary (full or part-time): Lumos Labs, Inc. **G. Morrison:** A. Employment/Salary (full or part-time): Lumos Labs, Inc..

Poster

751. Molecular Techniques

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 751.01/LLL65

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH, 1R01GM104948, 6926636

Title: Monomeric far-red and near-infrared fluorescent proteins for neuroimaging

Authors: *K. D. PIATKEVICH[1], E. JUNG[1], C. LINGHU[1], H.-J. SUK[1], D. PARK[1], B. SZABO[2], M. DROBIZHEV[3], F. CHEN[1], O. SHAMESH[1], E. S. BOYDEN[1];
[1]Media Lab., MIT, Cambridge, MA; [2]Dept. of Biol. Physics, Eotvos Univ., Budapest, Hungary; [3]Montana State Univ., Bozeman, MT

Abstract: Currently, most genetically encoded molecular tools used to study the brain are adopted from nature. However, the majority of naturally occurring proteins must to be optimized or reengineered before they can be adopted by researchers. We developed a simple approach that enables rapid directed molecular evolution of fluorescent proteins in mammalian cells to select for variants with several simultaneously optimized characteristics such as brightness, photostability, and cytotoxicity, in a single round of evolution, using a hierarchical screening strategy.
Using this method, we developed a pair of monomeric fluorescent proteins with far-red and near-infrared fluorescence spectra, named mfRFP and miRFP respectively, which are characterized by superior brightness, photostability and lower cytotoxicity in comparison to spectrally similar fluorescent proteins. Both mfRFP with excitation/emission maxima at 611/658 nm and miRFP with excitation/emission maxima at 674/703 nm correctly label fusion proteins in live cells and demonstrate excellent performance in neurons in culture and *in vivo*.
In contrast to earlier monomeric phytochrome-derived fluorescent probes, miRFP does not require co-expression of heme oxygenase 1 to enable its bright fluorescence in neurons in culture and *in vivo*. Furthermore, substantial overlap between the two-photon excitation spectra of

miRFP and blue-green fluorophores enables multicolor imaging using a single wavelength of excitation from a standard Ti-Sapphire laser. In addition, mfRFP expands the palette of fluorescent proteins compatible with our recently developed super-resolution imaging technique, protein retention expansion microscopy (proExM). We believe that mfRFP and miRFP will find broad applications as protein and cellular labels for *in vivo* imaging. Furthermore, directed evolution in mammalian cells may enable more rapid generation of synthetic biology tools for neuroscience than screens in bacteria.

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Poster

751. Molecular Techniques

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 751.02/LLL66

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIMH IRP

Title: Stability of lentivirus over hours at room temperature facilitates convection-enhanced delivery of DREADD expressing vectors into Rhesus monkey amygdala

Authors: *W. LERCHNER, M. A. G. ELDRIDGE, D. DEBORAH ROSE, V. DER MINASSIAN, V. COSTA, B. AVERBECK, B. J. RICHMOND;
LN/NIMH, NIH, Bethesda, MD

Abstract: To study the connection between behavior and its neural substrates we are using chemogenetic tools, such as DREADDs, in Rhesus monkeys to modulate neuronal activity. To carry out these experiments we would like to modulate the activity in volumes of tissue on the order of 10s of mm^3. Here we show results from slow infusions of lentivirus into the amygdala of rhesus monkeys using convection-enhanced delivery (CED). *Lentivirus stability.* We investigated the stability of lentivirus at room temperature (RT) with *in vitro* assays. Lentivirus expressing the hM_4Di-CFP DREADD under the control of the human synapsin promoter (Lenti-syn::hM4Di-CFP) was loaded into a syringe and expelled at six time-points between zero and six hours. Transduced T293 cells were harvested after 48 hours and used to determine viral titers. We also transduced T293 cells at the same time points with virus kept on ice. For the virus at RT the titer fell slightly between 0 and 30 minutes from 1.4x10E9 particles/ml to approximately 10E9 particles/ml but remained stable at from 30 minutes to 6 hours. The experiment was

repeated three times with consistent results. *DREADD expression in amygdala.* In a rhesus monkey, we made a single injection of 80 ul lenti-syn::hM4Di-CFP vector into the right amygdala at 1 ul/minute. In the same monkey we made four 20 ul injections into the left amygdala on a 2 x 2 mm grid, again at 1 ul/minute. For the injections into each amygdala a Hamilton syringe was filled with 90 ul of lentivirus before starting injections. Including loading, handling of the syringe and 10 minute wait times before removing the syringe after injections, the 80 ul injection into the right amygdala took approximately 100 minutes, and the four 20 ul injections into the left amygdala took a total of 140 min. Areas on histological sections where at least 50% of the neurons showed expression after post-mortem staining (penetrance) were outlined. Reconstruction showed that the single 80 ul injection filled a volume of ~45 mm^3 and the combined four 20 ul injections filled ~53 mm^3 with an average penetrance of >80%. Peak penetrance was close to 100% in approximately 30% of the tissue volume showing expression. The volume filled by the single injection was relatively uniform in distribution, whereas the four smaller injections covered a larger total volume of the amygdala, as there were some gaps with no cellular expression between injections**.** There was no decrease in coverage across the four individual injections on the left side, leading us to conclude that there is no degradation of virus transduction across the 4 injections (13, 12, 16, 12 mm^3). The beginnings of the first and last injections were separated by more than 100 min.

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Poster

751. Molecular Techniques

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 751.03/LLL67

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: A Novel cell based assay for screening Muscarinic M1 allosteric modulators

Authors: R. SUBRAMANIAN[1], S. EDULA[1], S. PETLU[1], M. NISSANKARARAO[1], M. SRIRANGAVARAM[1], V. MEKALA[1], *K. MUDIGONDA[2], R. NIROGI[1];
[1]Suven Life Sci. Ltd, Hyderabad, India; [2]Suven Life Sci., Hyderabad, India

Abstract: Alzheimer's disease (AD) is a debilitating neurodegenerative disorder afflicting millions of people. Cholinergic neurons degeneration and hypofunction are pathologies associated with Alzheimer's disease (AD). Earlier clinical studies has shown involvement of muscarinic acetylcholine subtype 1 (M_1) receptor in cognitive improvement and their clinical utility was limited by in-sufficient selectivity leading to cholinergic side effects. Muscarinic

acetylcholine receptors (mAChRs) mediate acetylcholine-induced neurotransmission and five mAChR subtypes (M1-M5) have been identified. Among them, M1 mAChR is widely expressed in the central nervous system and has been implicated in many physiological and pathological brain functions. In addition, M1 mAChR is postulated to be an important therapeutic target for AD and several other neurodegenerative diseases. Cholinergic side effects are due to conserved nature of the orthosteric site as a result it is difficult to identify selective M_1 receptor agonists. Allosteric modulation offer greater selectivity, physiological control retention and reduced over dose risk in comparison to traditional orthosteric compounds. Currently Calcium mobilization assay using Fluorescence Imaging Plate Reader (FLIPR); a kinetic assay which measures difference in Ca2+ levels in real time being widely used for Positive allosteric modulators of M1 receptor. Muscarinic receptors have biased signaling mechanism they primarily signal via Gαq and also couples to Gαs secondarily. Hence, we have developed cell based luciferase assay for screening the compounds which might mitigate the disadvantages of the existing method

Disclosures: **R. Subramanian:** A. Employment/Salary (full or part-time): Suven Life Sciences LTD. **S. Edula:** A. Employment/Salary (full or part-time): Suven Life Sciences LTD. **S. Petlu:** A. Employment/Salary (full or part-time): Suven Life Sciences LTD. **M. Nissankararao:** A. Employment/Salary (full or part-time): Suven Life Sciences LTD. **M. Srirangavaram:** A. Employment/Salary (full or part-time): Suven Life Sciences LTD. **V. Mekala:** A. Employment/Salary (full or part-time): Suven Life Sciences LTD. **K. Mudigonda:** A. Employment/Salary (full or part-time): Suven Life Sciences LTD. **R. Nirogi:** A. Employment/Salary (full or part-time): Suven Life Sciences LTD.

Poster

751. Molecular Techniques

Location: Halls B-H

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Program#/Poster#: 751.04/LLL68

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH EY024694

Kahn Neurotechnology

E. Matilda Ziegler foundation

Title: An experimental and bioinformatics pipeline for determining the contribution of alternative splicing to cell surface receptor diversity

Authors: *T. RAY[1], K. J. COCHRAN[2], O. FEDRIGO[3], M.-M. WANG[4], M.-X. HE[4], E. PARK[4], J. N. KAY[5];
[2]Computer Sci., [3]Duke Ctr. for Genomic and Computat. Biol., [1]Duke Univ., Durham, NC;
[4]Advanced Cell Diagnostics, Inc, Newark, CA; [5]Neurobio., Du, Durham, NC

Abstract: During development, neurons express an array of cell surface proteins that are instrumental to selective circuit wiring. These highly specific neural connections support cognitive abilities and perception. It has long been hypothesized that the number of cell surface proteins required for selective circuit wiring exceeds the number of protein coding genes in the genome. What mechanisms generate the required protein diversity? One possibility is alternative splicing of gene transcripts, which can greatly expand the functional coding capacity of the genome by generating multiple proteins from the same gene that differ in their receptor/ligand specificity. A few examples of this phenomenon are known (notably insect *Dscam*) but the extent to which alternative splicing diversifies neuronal receptor-ligand repertoires is unclear. A major obstacle to investigating this question is the lack of reliable methods to catalog and quantify the full-length transcripts generated by individual genes. We also lack methods to discern cell type-specific isoform expression patterns, limiting our ability to study protein isoform function in the context of neural circuit wiring. To overcome these obstacles, we developed a pipeline for identifying alternative splicing events relevant to forming the neural circuitry of mouse retina. First, we used traditional short read RNAseq datasets from retina to identify genes with significant alternative splicing within protein-coding regions. We identified 30 highly-expressed members of the epidermal growth factor and immunoglobulin superfamilies as candidate spliced cell-surface molecules. We developed a full-length cDNA-capture approach to sequence our 30 candidates at high depth on the PacBio RSII platform. This analysis was performed at various developmental time points. Custom bioinformatics algorithms were developed to identify and quantify known and novel isoforms. In this way we identified thousands of new splice isoforms of these 30 cell surface molecules, many of which are likely to change functional properties of the encoded proteins. We further identified novel genomic features including alternative promoter sites and alternative 5' and 3' UTRs, which might influence expression levels or subcellular localization of mRNA/protein. Finally, we used novel next generation *in situ* hybridization probes and assay technology to determine isoform specific spatial and temporal expression across retinal cell types. Our data reveal that isoform diversity for many cell surface receptors is greatly unresolved. How this diversity influences wiring of the nervous system is currently under investigation.

Disclosures: T. Ray: None. **K.J. Cochran:** None. **O. Fedrigo:** None. **M. Wang:** None. **M. He:** None. **E. Park:** None. **J.N. Kay:** None.

Poster

751. Molecular Techniques

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 751.05/LLL69

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH Grant R01 NS082553

NIH Grant R21 CA178965

NIH Grant R21 AR066931

Title: Phagocyte recruitment signals in apoptotic vesicles present in exosomal preparations

Authors: *V. V. DIDENKO;
Neurosurg., Baylor Col. of Med., Houston, TX

Abstract: Apoptotic bodies are the characteristic membrane blebs released by cells undergoing apoptosis. They carry “find-me” and “eat-me” signals which ensure migration of phagocytic cells to apoptotic sites and effective clearance of apoptotic debris. We developed a novel labeling approach for the detection and analysis of apoptotic bodies based on the composition of their cargo. Using this technique, we detected and investigated a population of small apoptotic bodies present in exosomal preparations isolated from various biological fluids by standard exosome purification methods. These apoptotic vesicles carry phagocyte recruitment signals and molecular markers identifying apoptotic mechanisms.
The new approach and the results of its application can be useful in studies of cell signaling in apoptosis and phagocytosis.

Disclosures: V.V. Didenko: None.

Poster

751. Molecular Techniques

Location: Halls B-H

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Program#/Poster#: 751.06/LLL70

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: Doxycycline (DOX)-controlled gating of gene expression in Rhesus monkey brain

Authors: *H. LAM, W. LERCHNER, M. A. G. ELDRIDGE, V. DER MINASSIAN, B. J. RICHMOND;
LN/NIMH, NIH, Bethesda, MD

Abstract: We are manipulating neural tissue using genetic techniques to probe the connection between neuronal function and behavior. To do this, we express proteins such as DREADDs that can affect the activity of neurons. By adding a means to gate gene expression, i.e. an 'on/off switch', it becomes possible to regulate expression of a gene of interest during desired intervals e.g. an shRNA to knock down a receptor or the overexpression of a protein affecting neural activity. In rodents, this has been done using DOX systems.
Here we test 4 different potentially useful DOX-inducible systems using lentivirus in cell culture and in the monkey brain:
1) A two-vector DOX-ON system using neuron specific rtTA expression (DOX^{ON}-2),
2) A two-vector DOX-OFF system using neuron specific tTA expression (DOX^{OFF}-2),
3) A one-vector DOX-ON system using neuron specific rtTA expression (DOX^{ON}-1),
4) A one-vector DOX-ON system with auto-regulatory loop using neuron specific tTS^{kid} expression (DOX^{AUTO}-1).
All systems were initially tested with the mCherry-2A-dsGFP reporter gene to visualize short-term expression via destabilized GFP (2 hours half-life) and long-term expression including axonal terminals via mCherry. *In vitro* studies (fluorescent protein imaging in cell culture and Western Blots) revealed that all systems produced on/off switching and strong induction with the exception of *DOX^{AUTO}-1* for which only weak expression was detected after prolonged treatment with DOX. Replacing mCherry-2AdsGFP with the hM4Di-CFP DREADD yielded similar results.
We then tested the constructs in monkeys under the following conditions:
Monkey A: No DOX for 4 weeks.
Monkey B: No DOX for 4 weeks, then 10mg/kg DOX daily for 4 days.
Monkey C: 10mg/kg DOX daily for 4 weeks, then no DOX for 2 days.
DOX^{AUTO}-1 performed as expected with no detectable expression in the absence of DOX and strong expression in the presence of DOX; after 2 days of DOX removal following prolonged treatment, only mCherry persisted in some cells and projections. *DOX^{OFF}-2* performed more or less as expected with strong expression both in the absence of DOX and after 2 days of DOX removal following prolonged treatment; however, in the prolonged presence of DOX, we did observe some weak expression of mCherry. Results from the other systems remain inconclusive at this time and are being tested further. Our findings demonstrate that DOX-inducible systems (single and dual vector) are capable of regulating gene expression in the monkey brain.

Disclosures: H. Lam: None. **W. Lerchner:** None. **M.A.G. Eldridge:** None. **V. Der Minassian:** None. **B.J. Richmond:** None.

Poster

751. Molecular Techniques

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Program#/Poster#: 751.07/MMM1

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: Autoradiography as a universal tool to measure quantitative receptor density and G protein-coupled receptor activation

Authors: J. RYTKÖNEN, T. PARKKARI, J. PUOLIVÄLI, S. ALASTALO, *O. M. KONTKANEN, P. J. SWEENEY, A. NURMI, T. HUHTALA;
Charles River Discovery, Kuopio, Finland

Abstract: Autoradiography is a powerful technique that can be applied to study receptor density and activation of G protein-coupled receptors (GPCRs) by novel pharmacological compounds in the brain. These methodologies are easily applicable to various disease models, and combining them with behavioral readouts allows a versatile evaluation of pathophysiology of the CNS disorders and mechanisms of action of novel therapies. GPCRs are involved in a wide variety of physiological processes, including regulation of behavior and mood. They activate intercellular signal transduction pathways and cellular responses. Cannabinoid, serotonin, dopamine, GABAB, and metabotropic glutamate receptors are among neurologically interesting GPCR targets. Ligand binding to GPCRs induces an interaction of the receptor with G protein that stimulates the release of GDP simultaneously with the exchange of GTP. ^{35}S-GTPγS autoradiography has been applied to study receptor activation after ligand binding to GPCRs of Gi and Gs types. In this study, we analyzed changes in the receptor density in chronic social defeat (CSD) model that has robust depression-like behavioral endpoints. To assess receptor density alterations, tritiated ligands to dopamine receptors 1 and 2, serotonin transporters and cannabinoid receptor 1 (CB1) were used and Bmax values of corresponding ligands were determined in relevant brain regions. Changes in Bmax values correlate with alterations in the number of available receptors sites and which may correlate with the phase or severity of the disease. We also determined the dose-response relationship of GPCR activation by the CB1 agonist HU-210 by detecting consumption of GTPγ^{35}S in the somatomotor cortex, cingulate cortex, striatum, globus pallidus and substantia nigra in mice. The potency (half maximal effective concentration, EC50) as well as efficacy (Emax) of interaction of HU-210 with CB1 was calculated from the obtained dose-response curves. The current examples demonstrate how a combination of assays can be utilized to advance our understanding of the disease state and measure effects of pharmacological treatments. Also, applied digital scintillation autoradiography enables quantitative and real-time imaging of tritiated samples within hours, compared to minimum of several weeks of exposure time required to phosphoscreens or films. This accelerates method development and analysis substantially. As a summary, the combination

of receptor and functional autoradiography offers a powerful tool to comprehensively measure changes in disease models or responses to novel molecules.

Disclosures: **J. Rytkönen:** None. **T. Parkkari:** None. **J. Puoliväli:** None. **S. Alastalo:** None. **O.M. Kontkanen:** None. **P.J. Sweeney:** None. **A. Nurmi:** None. **T. Huhtala:** None.

Poster

751. Molecular Techniques

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Program#/Poster#: 751.08/MMM2

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIGM-115042

NIMH-106245

NSF-1002410

NSF-1137725

Title: Fret for measuring distances of connexin containing gap junctions

Authors: ***H. DECKER**[1,2,3], N. MARTINEZ-RIVERA[2], S. LIN[4], M. BROWNE[5], E. ROSA-MOLINAR[1,2,3];
[1]Microscopy and Analytical Imaging Lab., [2]Biol. Imaging Group, [3]Neurosci. Grad. Program, Univ. of Kansas, Lawrence, KS; [4]Andor Technology, Inc., Belfast, United Kingdom; [5]Andor Technologies, Belfast, United Kingdom

Abstract: In this study, we characterized a developmental 2D model approach using Förster Resonance Energy Transfer (FRET) to determine the spatial distance of connexin-containing gap-junctions within a stimulated synapse. FRET has long been used as a method to increase fluorescence resolution, the transfer of energy from a donor to an acceptor which generally occurs between 10-100Å, allowing one to determine the relative distance between the donor molecular and the acceptor molecule. In this study, we focused on a specific method to determine FRET, acceptor photo-bleaching. This method occurs when the acceptor fluorophore is completely photo-bleached or destroyed. If the FRET pair is close enough, there will be an increase in donor fluorescence. In order to establish that FRET is possible on a structure as small as a connexin-containing-gap junction, we immuno-labeled GFP-tagged Connexin 35 expressing cells with anti-connexin 35 and photobleached the connexin. This strategy a) allowed us to characterize the methodology necessary to determine FRET of the connexin, b) assisted in the

development of a 2D model that allows the precise testing of multiple parameters, and c) increased throughput. Once this model was established, cells were labeled with the fixable plasma membrane dye, mCling647N, and a connexin 35 antibody for FRET analysis. The FRET efficiency for each Connexin containing gap junction was calculated using the acceptor photo-bleaching method established by Kenworthy and Edidin (1997; J Cell Biol, 142:69-84). Future work will include the use of different sizes of fluoronanogold conjugated antibodies for correlative microscopy in order to determine accurate distance between connexins.

Disclosures: H. Decker: None. **N. Martinez-Rivera:** None. **S. Lin:** None. **M. Browne:** None. **E. Rosa-Molinar:** None.

Poster

751. Molecular Techniques

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Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: Project GENCODYS No. 241995

Project EUROSPIN No. 242498

Project SYNSYS No. 242167

Title: Using the halotag technology to investigate the turnover of psd-95 at single synapses.

Authors: *M. KRATSCHKE[1], D. FRICKER[1], N. H. KOMIYAMA[2], S. G. N. GRANT[2];
[2]Ctr. for Clin. Brain Sci., [1]Univ. of Edinburgh, Edinburgh, United Kingdom

Abstract: Existing methods for analysing synaptic protein turnover are unable to provide adequate information about synthesis and degradation, particularly at single synapses. PSD95 is an abundant scaffolding protein found in the postsynaptic densities of excitatory synapses throughout the mammalian brain, and plays a critical role in innate and learned behaviours. PSD95 assembles with many other proteins into postsynaptic supercomplexes (Frank et al, 2016) that are then organised into nanoclusters that comprise the postsynaptic density of excitatory synapses (Broadhead et al, 2016).
The HaloTag consists of a modified bacterial haloalkane dehalogenase protein domain that covalently binds synthetic chloroalkane ligands that can be labelled with fluorescent and affinity moieties. Hence, cells expressing proteins fused to the HaloTag can be used to study protein levels, complexes and turnover using the different ligands.
We have adapted this method to study synaptic proteins by fusing the HaloTag to endogenous

PSD95 using gene targeting in the mouse. Using primary cultured neurons from PSD95-HaloTag mice we have shown that the HaloTag does not alter the expression level of PSD95, nor its normal assembly into 1.5 MDa supercomplexes. Next we show that ligands coupled to fluorophores label virtually 100% of PSD95 and individual postsynaptic puncta can be visualised with confocal microscopy. Using a pulse-chase paradigm, where one ligand (R110) is used to label all existing PSD95 first, we can then label any newly synthesised PSD95 using a second ligand (TMR). We can thus identify and characterise subpopulations of PSD95 and thereby analyse its synthesis and degradation. We find that PSD95 has a half-life of 33.2 hours ±2.5 (SEM) at single synapses, consistent with previous literature (El-Husseini et al, 2002). Furthermore, we have observed marked synaptic heterogeneity in PSD95 turnover, suggesting some synapses have more stable PSD95 complexes.
We also applied drugs known to modulate protein turnover and neuronal activity and analysed their effect on PSD95 turnover. We found that the proteasome inhibitor Lactacystin (1μM) not only significantly reduces PSD95 degradation, but concomitantly inhibits PSD95 synthesis over a 24-hour time period. This method offers a unique way of investigating the turnover of a specific, tagged protein. The effects of various modulatory drugs on PSD95 turnover are currently being investigated.
References: Broadhead et al. (2016) Nat Sci Rep, 6:24626
Frank et al. (2016) Nat Comm 7:11264
El-Husseini et al. (2002) Cell, 108:849-863

Disclosures: **M. Kratschke:** None. **D. Fricker:** None. **N.H. Komiyama:** None. **S.G.N. Grant:** None.

Poster

751. Molecular Techniques

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Program#/Poster#: 751.10/MMM4

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: SSTF-BA1301003

Title: Analysis of mitochondrial transport dynamics in axons

Authors: *K.-T. MIN;
UNIST, Ulsan, Korea, Republic of

Abstract: The polarized structure and extremely long neurites pose a unique challenge for neurons to distribute mitochondria to the appropriate locations. Hence, proper transport of

mitochondria to the appropriate locations is especially important for neuronal functions. Consequently, defective mitochondrial transport can have deleterious effects on neuronal functions and survival. It is widely accepted that mitochondria move from the cell body to axon terminals and vice versa. Although mitochondria are known to move bi-directionally from the cell body to axon terminals and vice versa, the dynamic pattern of mitochondrial transport in the entire length of axons for an extended period of time have not been thoroughly examined. Using the photo-switchable fluorescent protein dendra-2 targeted to mitochondria, we tracked individual mitochondria in the whole axon. Surprisingly, we find mitochondria that originated from the axon terminals traveling in the retrograde direction never reach at the cell body. Retrogradely moving mitochondria failed to enter the cell body due to fusion to existing mitochondria or pausing in the middle of axons, while anterogradely moving mitochondria reach the axon terminals with less interruption. In addition, the speed of mitochondrial transport varies along regions with different densities of stationary mitochondria. Stationary mitochondria were present at higher density in axon proximal to the cell body, although inter-mitochondrial spacing is random following Gumble-like distribution. Furthermore, we derived a mathematical model using the Fokker- Planck equation to characterize the features of mitochondria movement, which was successfully adopted to determine altered mitochondrial transport in axons overexpressing parkin. Our analysis and model provide new insights into the dynamics of mitochondria transport in axons of normal or unhealthy neurons.

Disclosures: K. Min: None.

Poster

751. Molecular Techniques

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Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: UNAM-PAPIIT-DGAPA Key: IT201112

PAL Program UNAM-Facultad de Química. Key: 3000/3070

Title: Immunolocalization of melatonin and its analogue M3C in testis of rat

Authors: *E. N. RODRIGUEZ;
Univ. Nacional Autónoma De México, Mexico City, Mexico

Abstract: Melatonin (MT) (N-Acetyl5-metoxytryptamine) is a lipophilic hormone produced by the pineal gland during the scotophase. The MT has activities in regulating biological rhythms

neuroimmunologic, antioxidant, gastroprotective, antiinflammatory, anxiolytic-antidepressant-like, antiproliferative and reproduction. In humans, (>60 years old) the concentration of MT and decreases less than that at puberty 80%. Currently, there is a therapeutic use exogenous MT without known toxicity and side effects. Debeliuk et al. (1969) showed that 300 µg of MT for 30 days, reduces the weight of the testes and affects the production of testosterone in rats. Redins et al., (2002) administered, 100 µg of MT (22 days), show a level ultrastructure damage in mouse Leydig cells. In 2011, Tuncer et al., reported a tubular degeneration, edema and obstruction of seminiferous tubules, with 3 mg/kg of MT (30 days) in rats. On this basis, we made studies with MT and it´s analogue 1-N-substituted (M3C) and to know if the testicular seminiferous tubule cell structure (ST) have any changes in rat by chronic administration for both substances, and demonstrate the immunolocalization of TM in this structure in male rats. Independent treatment with 300 µg of two substances (30 days) was applied. Post-treatment, the animals were perfused with buffered formaldehyde with saline solution and the testes for histopathological study (hematoxylin-eosin staining) and immunolocalization of MT with specific antibody anti-melatonin. The MT and M3C were located in ERA. Supported by UNAM-PAPIIT-DGAPA Key: IT201112 PAL Program UNAM-Facultad de Química. Key: 3000/3070

Disclosures: E.N. Rodriguez: None.

Poster

751. Molecular Techniques

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Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: CIHR grant

CNS grant

Title: Deciphering the cellular and physiological actions of cell-penetrating pepducins affecting NTS1 signaling

Authors: *R. L. BROUILLETTE, C. MONA, É. BESSERER-OFFROY, S. LAVENUS, J. CÔTÉ, M. SOUSBIE, J.-M. LONGPRÉ, R. LEDUC, M. GRANDBOIS, É. MARSAULT, P. SARRET;
Pharmacologie-Physiologie, Univ. De Sherbrooke, Sherbrooke, QC, Canada

Abstract: G protein-coupled receptors (GPCRs) have proven to be effective targets in drug discovery. However, many believe that the therapeutic potential of this protein class remains

largely untapped. In recent years, new strategies have been developed to more fully exploit this potential, including cell-penetrating peptides known as pepducins. These lipidated peptides are able to translocate across the cell membrane and target the intracellular loop of a GPCR of interest, interacting at the receptor-effector interface. There, pepducins have been shown to act as allosteric agonists as well as positive or negative allosteric modulators of their cognate receptor. In the present study, we generated a series of pepducins targeting the first intracellular loop of the human neurotensin receptor 1 (hNTS1), a class A GPCR shown to mediate many of the physiological effects of the neurotensin (NT) tridecapeptide including analgesia, hypotension, and hypothermia. In CHO-K1 cells stably expressing hNTS1, we investigated the pepducins' effect on the orthosteric binding of ^{125}I-labeled NT. We also used BRET-based biosensors to verify the pepducins' ability to engage the Gαq, Gαi1, GαoA, and Gα13 pathways, as well as to recruit β-arrestins 1 and 2. Furthermore, we monitored pepducin-induced changes in whole-cell integrated response by surface plasmon resonance (SPR) in the same cell-type. Finally, in *in vivo* and *ex vivo* experiments, respectively, we evaluated the pepducins' ability to trigger blood pressure changes in carotid artery-cannulated rats and to induce smooth muscle relaxation of pre-contracted rat ileum tissue in isolated organ baths. Our *in vitro* results showed a partial decrease in [^{125}I]-NT binding and only minimal G protein-dependent and G protein-independent signaling pathway activation in agonist mode at concentrations of 10^{-5} M. Intriguingly, the noninvasive label-free whole-cell sensing assays revealed the existence of distinct and dissimilar SPR profiles following incubation with our pepducin series. Moreover, when systemically injected in rats, NTS1-selective pepducins were shown to exert more potent hypotensive effects at 55 nmol/kg than NT(8-13) at the same dose, and to induce a sustained mean drop in blood pressure of 30 mm Hg. We also observed ileum relaxation at high pepducin concentrations of 10^{-5} M. In summary, our results show that our set of pepducins participates in cellular and physiological effects linked to NTS1 activation, and may thus constitute a promising avenue of research into other properties of the neurotensinergic system, such as NT-induced analgesia.

Disclosures: **R.L. Brouillette:** None. **C. Mona:** None. **É. Besserer-Offroy:** None. **S. Lavenus:** None. **J. Côté:** None. **M. Sousbie:** None. **J. Longpré:** None. **R. Leduc:** None. **M. Grandbois:** None. **É. Marsault:** None. **P. Sarret:** None.

Poster

751. Molecular Techniques

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Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: Hilda and Preston Davis Foundation Postdoctoral Fellowship in Eating Disorder Research

Title: Light-inducible cre recombinase: using LOV domains to create a single-molecule light activated DNA recombinase enzyme

Authors: *D. M. OPLAND[1], D. S. ABRAMOV[2], C. W. BOND[2], E. P. FOSCUE[2], R. J. DILEONE[2];
[1]Mol. Psychiatry, [2]Yale Univ., New Haven, CT

Abstract: As a primary tool for controlled gene expression, DNA recombination is widely used throughout neuroscience. Gated control of recombinase activity has several current applications ranging from specific developmental studies as well as in defining the time window for tagging active neural ensembles. Current inducible gene expression systems rely on pharmacological ligands to gate DNA recombinase activity. The two most ubiquitous systems use either the antibiotic tetracycline or the estrogen receptor antagonist tamoxifen for control of recombinase activity. With both systems, the innate pharmacokinetics of the effector ligand defines the time-window of enzyme action, typically lasting hours to days. While this level of temporal specificity is sufficient for some implementations, improved temporal dynamics will broaden the use and impact of these approaches in neuroscience. We are developing and testing an alternative inducible recombinase system in which light controls the temporal dynamics of enzyme activity to increase the precision of controlled gene expression. This system is reliant on fusion proteins of Cre recombinase and the light-responsive subunit of the phototropin 1 gene (the LOV domain). In the absence of light, the LOV domain remains in a tightly bound formation preventing Cre recombination. However, when illuminated with blue (488 nm) light, the LOV domain unfurls and permits recombinase activity. Initial LOV-Cre fusion variants were created by varying the length of the amino acid tether connecting the protein domains and show moderate dark-state inhibition of Cre activity. Light stimulation of LOV-Cre (30 - 180 minutes) in an in vitro model system show a small increase in recombinase activity demonstrating proof of principle that this strategy can be leveraged to create a functional light-inducible DNA recombinase enzyme. Current work is focused on increasing the dynamic range of the early LOV-Cre fusion proteins via rational amino acid substitutions [Strickland D et al (2008) PNAS Aug 5; 105(31):10709-14] and to extending testing to in vivo models of recombinase mediated gene expression.

Disclosures: D.M. Opland: None. **D.S. Abramov:** None. **C.W. Bond:** None. **E.P. Foscue:** None. **R.J. DiLeone:** None.

Poster

751. Molecular Techniques

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Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: Graduate Fellowship, The Rockefeller University

Title: Using CLIP to measure alternative polyadenylation in specific neurons

Authors: *S. JEREB, H.-W. HWANG, R. B. DARNELL;
The Rockefeller Univ., New York, NY

Abstract: Alternative polyadenylation is a process where different ends to an mRNA transcript are selected. These alternative ends contain regulatory 3′ untranslated regions (3'UTRs) and/or coding sequences. Recent evidence has shown that mammalian and fly brains express particularly long 3'UTR isoforms compared to other tissues. Alternative polyadenylation has been implicated in the regulation of subcellular protein and mRNA localization, translational regulation and mRNA stability. However, little is known about the *in vivo* functional roles of alternative polyadenylation in neurons. To form a more comprehensive view of alternative polyadenylation in the brain, we set out to determine the 3'UTR isoform diversity in two types of functionally and morphologically distinct neurons in the mouse cerebellum: excitatory granule neurons and inhibitory Purkinje neurons. By expressing GFP-tagged poly(A) binding protein cytoplasmic 1 (Pabpc1) in each cell type separately, we were able to sequence 3' mRNA ends using anti-GFP crosslinking immunoprecipitation (CLIP). Annotation of 3' ends revealed extensive isoform diversity between Purkinje and granule neurons with 1041 genes displaying differential 3'UTR isoform usage. Notably, Purkinje neurons tended to express fewer long 3'UTR isoforms compared to granule neurons. Moreover, it has been reported that alternative polyadenylation changes during embryonic development of an organism. To explore the role of alternative polyadenylation during neuronal development in more detail we focused on the development of cerebellar granule neurons. We annotated 3' ends in proliferating precursors of granule neurons and in mature granule neurons. 852 genes displayed differential 3'UTR isoform usage between the two developmental time points, most of the genes expressed a longer isoform in mature granule neurons. Interestingly, genes involved in ubiquitination were enriched both among genes that change 3'UTR isoform expression during development as well as those that differ in 3'UTR isoform expression between Purkinje and granule neurons. We are currently exploring the functional roles of the mRNAs that show 3'UTR isoform shift during granule neuron development.

Disclosures: **S. Jereb:** None. **H. Hwang:** None. **R.B. Darnell:** None.

Poster

751. Molecular Techniques

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Program#/Poster#: 751.15/MMM9

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: Brain Research New Zealand Postdoctoral Fellowship

Title: Probing synaptic plasticity: a single cell RNA sequencing approach

Authors: *H. J. MCQUILLAN[1], P. J. GUILFORD[1], R. C. DAY[1], B. J. SNOW[2], J. N. J. REYNOLDS[1];
[1]Univ. of Otago, Dunedin, New Zealand; [2]Auckland District Hlth. Board, Auckland, New Zealand

Abstract: Much of our current knowledge about synaptic plasticity has stemmed from electrophysiological recordings from single cells. However, such an approach, particularly *in vivo*, is inherently low yield and time consuming. Single-cell RNA sequencing represents an alternative approach with great potential, largely due to the recent development of several new RNA sequencing methodologies.
Single cell resolution lends itself well to studies of complex heterogeneous areas of the brain such as the striatum. In order to accurately identify single neurons not readily distinguishable by conventional microscopy, we first established a rapid (10 minute) labeling method by immunofluorescence on fresh frozen 12 μm sections. Antigens specific to the direct spiny neurons (substance P), indirect spiny neurons (enkephalin) and the cholinergic interneurons (choline acetyltransferase) of the striatum were able to be detected, while maintaining RNA integrity. Laser capture microdissection was then used to isolate neurons as either single cells or pools of single cell types. The Smart-seq 2 methodology for transcriptomic analysis was used due to the high level of cDNA yield, sensitivity, accuracy and full length coverage afforded by this approach.
We present an evaluation of the quality of the transcriptomic data obtained from the striatum by combining immunohistochemical labelling and laser capture isolation with the Smart-seq2 RNA sequencing approach. We explore the application of the approach across different paradigms of synaptic plasticity and the potential application of this approach to the investigation of neurological disorders.

Disclosures: **H.J. McQuillan:** None. **P.J. Guilford:** None. **R.C. Day:** None. **B.J. Snow:** None. **J.N.J. Reynolds:** None.

Poster

751. Molecular Techniques

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Program#/Poster#: 751.16/MMM10

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: Novel approaches to visualise the expression, localization, and signaling of TLR4

Authors: *J. THOMAS[1,3], S. MUSTAFA[1,3], C. ASHWOOD[2,4], L. M. PARKER[2,4], N. H. PACKER[2,4], M. R. HUTCHINSON[1,3];
[1]Ctr. For Nanoscale Biophotonics, Adelaide, Australia; [2]Ctr. For Nanoscale Biophotonics, Sydney, Australia; [3]Univ. of Adelaide, Adelaide, Australia; [4]Macquarie Univ., Sydney, Australia

Abstract: Toll-Like receptors (TLR) are classically viewed as a type of pattern recognition receptor involved in peripheral innate immune responses, leading to the upregulation, synthesis and secretion of pro-inflammatory mediators. However, it is now becoming apparent that TLR4 is involved in more than just innate immunity, with major links to the etiology of opioid tolerance, chronic pain, addiction, itch, and depression. Current techniques that are used to study the localization and signaling of TLR4 have been optimized for the study of classical peripheral immunity. In the pursuit of a more comprehensive understanding of the involvement of TLR4 in these pathologies, it has become apparent there are limitations to applying the current techniques for studying TLR4 in CNS tissue. Here we aim to develop new methods for identifying TLR4 protein and mRNA localization, and TLR4 signaling cascades, with an influence on optimising for CNS tissues. A transdisciplinary approach has been applied to identify the problems and source the experimental solutions to this TLR4 measurement and visualisation challenge. A novel in situ hybridization approach was developed using the application of photostable nanoparticles to visualize the localization and expression of TLR4 mRNA. This technique demonstrates significant TLR4 specificity in both cells and tissue samples with exceptional photostability compared with traditional fluorescent methods. The challenges with imaging and quantifying TLR4 protein have also been explored, with the non-specificity of commercially available antibodies highlighted. Additionally, development of a novel workflow for targeted mass spectrometric quantification of TLR4 has demonstrated surprising challenges associated with the detection and measurement of TLR4. Finally, the creation of novel fluorescent constructs of TLR4 has allowed visualization of cellular localization of TLR4 and the potential use in bioluminescent resonance energy transfer assays for real time quantification of TLR4 activation. The collective use of these tools will be presented in the context of chronic pain and opioid exposure.

Disclosures: **J. Thomas:** None. **S. Mustafa:** None. **C. Ashwood:** None. **L.M. Parker:** None. **N.H. Packer:** None. **M.R. Hutchinson:** None.

Poster

751. Molecular Techniques

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 751.17/MMM11

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NBIA Grant

Title: Novel therapeutic strategies in nbia: a gene therapy approach for pla2g6-associated neurodegeneration

Authors: *S. CUKA[1], J. NG[2], K. SEINO[5], M. HUGHES[3], S. WADDINGTON[2], M. KURIAN[4], A. RAHIM[3];
[1]UCL Sch. of Pharm., UCL, Kent, United Kingdom; [2]Inst. for Women's Hlth., [3]Sch. of Pharm., [4]Inst. of Child Hlth., Univ. Col. London, London, United Kingdom; [5]Inst. of Med. Sci., St Marianna Univ. Sch. of Med., Kanagawa, Japan

Abstract: Infantile neuroaxonal dystrophy (INAD) is a debilitating, intractable and ultimately lethal neurodegenerative disorder. It is caused by mutations in the PLA2G6 gene that encodes for phospholipase A. Patients present neurodegeneration-associated symptoms between six months and three years of age. Severe spasticity, progressive cognitive decline, and visual impairment typically result in death during the first decade. There is no disease-modifying treatment available and palliative care focuses on quality of life. There is an overwhelming need to develop novel therapies to treat INAD patients.
We aim to conduct a preclinical AAV-mediated gene therapy study to prevent neurodegeneration and rescue a mouse model of INAD from premature death. We will use recombinant adeno-associated virus serotype 9 vector (AAV9) to deliver therapeutic human PLA2G6 gene to the neonatal INAD mouse CNS via either intracranial or intravenous administration. The human PLA2G6 and control GFP gene were cloned into an AAV9 backbone plasmid, driven by the synapsin-I promoter and used to produce high titre viral preparations. Furthermore, the INAD mouse model recapitulates many features of the human phenotype. Investigating indices of neuropathology will not only provide us with readouts against which to gauge therapeutic efficacy but also increase our understanding of the underlying disease mechanisms.

Disclosures: **S. Cuka:** None. **J. Ng:** None. **K. Seino:** None. **M. Hughes:** None. **S. Waddington:** None. **M. Kurian:** None. **A. Rahim:** None.

Poster

751. Molecular Techniques

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 751.18/MMM12

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: Non-biased miRNA purification from clinical samples stored in TRIzol.

Authors: S. FORMAN, D. CABAYA, *J. A. VALADEZ, X. JIA;
Zymo Res. Corp, Irvine, CA

Abstract: Organic extraction, with TRIzol, TRI Reagent or similar, is often a method of choice for pathogen inactivation and sample stabilization and thus plays a significant role in clinical sample processing prior to nucleic acid analysis. However, the organic extraction methods were shown to introduce significant bias, specifically into the small RNA (miRNA) recovery. We have developed a non-biased method that allows for binding nucleic acids, including miRNAs, directly, i.e., without phase separation or precipitation, following the organic extraction step. We have compared the miRNA profiles obtained through next generation sequencing as well as a hybridization approach with an existing non-biased "double extraction" protocol (Ambion) and confirmed the non-biased purification results.This novel "direct nucleic acid binding" approach is fully automatable and allows the use of organic extraction in high-throughput applications.

Disclosures: S. Forman: None. **D. Cabaya:** None. **J.A. Valadez:** None. **X. Jia:** None.

Poster

751. Molecular Techniques

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 751.19/MMM13

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: 1R01DK097519

Title: miR-126 mediates Tβ4-induced neurovascular remodeling in diabetic peripheral neuropathy

Authors: *X. LU;
Henry Ford Hosp., Detroit, MI

Abstract: Background: MicroRNAs (miRs) play pivotal roles in regulating post transcriptional gene translation and mediating biological function. We previously demonstrated that treatment of diabetic mice with thymosin-β4 (Tβ4) significantly ameliorated neurological dysfunction caused by diabetic peripheral neuropathy, which associated with Angiopoietin1 (Ang1) promotes neurovascular function. In this study, we investigated whether miR-126 is involved in the effect of Tβ4 on Ang1 expression and neurovascular remodeling.
Methods and Results: Male BKS.Cg-m+/+Leprdb/J (db/db) mice (n=10) were treated with Tβ4 (30 mg/kg, i.p. daily) for 8 consecutive weeks, and db/db mice (n=10) were used as a control group. Compared with non-diabetic mice (dm), diabetic mice (db/db) exhibited significant reduction of miR-126 expression in peripheral serum samples (0.4±0. 2 vs.1.0±0.1, $p<0.05$) and sciatic nerve tissue (0.3±0.07 vs.1.0±0.07, $p<0.05$). Treatment of db/db mice with Tβ4 significantly upregulated miR-126 expression in serum samples (1.2±0.1 vs. 0.4±0.2 in saline, $p<0.05$) and sciatic nerve tissue (0.8±0.02 vs.0.3±0.07 in saline, $p<0.05$), measured by quantitative RT- PCR. Western blot analysis revealed that diabetes decreased levels of Ang1 (0.4±0.07 vs.1.0±0.1, in dm mice, $p<0.05$) and AKT activity (pAKT, 0.3±0.03 vs.1.0±0.04 in dm mice, $p<0.05$) in sciatic nerve tissue compared to dm mice. However, Tβ4 treatment overcame the effect of diabetes on these proteins (Ang1, 0.9±0.06 vs. 0.4±0.07 in saline, pAKT 0.7±0.05 vs.0.3±0.03 in saline, $p<0.05$). In vitro, treatment of mouse dermal endothelial cells (MDE) and DRG neurons with Tβ4 significantly increased capillary-like tube formation (1.1±0.06 vs. 0.6±0.06 in control, $p<0.05$) and axonal outgrowth (1,231±75.1*u*m vs.1,008±77.2*u*m in control, $p<0.05$), respectively, under hyperglycemia conditions, whereas attenuation of endogenous miR-126 by siRNA against miR-126 abolished the effect of Tβ4 on endothelial cell capillary tube formation (0.7±0.07 vs.1.0±0.08 in scramble control, $p<0.05$) and DRG neuron axonal outgrowth (1,079±42.5*u*m vs.1,235±48.6*u*m in scramble control, $p<0.05$). Quantitative RT-PCR analysis showed that Tβ4 increased Ang1 expression in MDE (2.35±0.1 vs.1.06±0.2 in control, $p<0.05$) under hyperglycemia and that blockage of miR-126 suppressed Tβ4-increased Ang1 expression (0.49±0.2 vs. 1.47±0.1 in scramble control, $p<0.05$).
Conclusion: These data suggest that Ang/Tie2 and PI3K/Akt signaling pathways mediate the therapeutic effect of Tβ4 on vascular remodeling and axonal outgrowth. miR-126 regulates Ang1 expression under diabetic conditions with and without treatment of Tβ4.

Disclosures: X. Lu: None.

Poster

751. Molecular Techniques

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 751.20/MMM14

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH Grant R21GM114852

miBrain initiative

Title: Second-generation brainbow adeno-associated virus with improved labeling brightness, color diversity and neuronal subtype identity

Authors: F. LU, A. STOLZ, *D. CAI, J. S. STECHER, D. ROOSSIEN, Z. HALL;
Cell and Developmental Biol., Univ. of Michigan, Ann Arbor, MI

Abstract: Creating random colors in individual cells, the Brainbow technology allows labeling and distinguishing intermingled neighboring neurons in the same brain. The introduction of Brainbow labeling by adeno-associated virus (AAV), a safe and chronic gene delivery vector broadly used in neuroscience, expanded the application potentials of Brainbow. The first generation of Brainbow AAV system delivers 4 spectrally distinguishable fluorescent proteins (FP) by mixing two viral species, each of which packaged two FPs and upon Cre recombination, a non-fluorescent or either FP (total of 3) outcome will be generated. Therefore, a 1:1 mix of these AAVs allows up to (3x3-1) eight possible color combinations. The first generation of Brainbow AAV also targets FPs to the cytoplasmic membrane to homogenously label even the finest axonal and dendritic processes. However, immunofluorescence amplification is often required to increase the labeling brightness, which costs additional experimental procedures and time. We created the second generation of Brainbow AAV to overcome the labeling brightness limitation and brought additional improvements including increased labeling color diversity and neuronal subtype identity. We further reduced the AAV packaging content to one FP species per virus, which allowed us introducing FPs in tandem copies to increase native fluorescent intensity. We also redesigned the recombination scheme that either an ON or an OFF outcome will be created after Cre recombination. Therefore, mixing equal amount of 4 spectrally distinguishable FPs will generate (2^4-1) fifteen possible color combinations. Finally, a new set of Brainbow AAVs are created to allow labeling of two neuronal subtypes in the same animal, in which Cre and Flp recombinases are expressed in two distinct population of neurons.

Disclosures: F. Lu: None. **A. Stolz:** None. **D. Cai:** None. **J.S. Stecher:** None. **D. Roossien:** None. **Z. Hall:** None.

Poster

751. Molecular Techniques

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 751.21/MMM15

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: Novel sample preservation for non-biased recovery and detection of miRNA markers in clinical samples.

Authors: S. FORMAN, L. BASILIO, *A. D. CLAUSEN, X. JIA;
Zymo Res. Corp., Irvine, CA

Abstract: Sample collection and preservation is crucial for accurate and non-biased nucleic acid analysis. We have identified major biases in detection of miRNA species implicated in neurodegenerative disease. We tested and compared collection devices, reagents and purification workflows in their ability to preserve and allow for efficient recovery of nucleic acids, specifically miRNAs from whole blood. To evaluate the miRNA expression in clinical samples we used the next generation sequencing (Illumina) and hybridization-based analysis (Nanostring). The expression profile comparison shows high correlation between the two detection methods. However, our study identifies workflows and reagents that result in absence or underrepresentation of miRNA species, including those implicated in neurodegenerative disease (e.g., miR-126; Parkinson's disease).

Disclosures: S. Forman: None. **L. Basilio:** None. **A.D. Clausen:** None. **X. Jia:** None.

Poster

751. Molecular Techniques

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Program#/Poster#: 751.22/MMM16

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH R01 AG036400

AARC DHS Award FY 2012 to PDC

Title: Novel method to ascertain chromatin accessibility at specific genomic loci from frozen brain homogenates and laser capture microdissected defined cells

Authors: E. DELVAUX[1], D. MASTROENI[1,2], J. NOLZ[1], *P. D. COLEMAN[1,2];
[1]ASU-Banner Neurodegenerative Res. Ctr., Tempe, AZ; [2]Banner Sun Hlth. Res. Inst., Sun City, AZ

Abstract: Numerous epigenetic mechanisms converge to modulate chromatin structure and many methods exist to delineate this structure. DNase I digestion is a conventional method used. However, a major limitation of DNase I based methods is the susceptibility of DNase I to inhibition by actin, a component of many cells and tissues. Recently, a protocol was developed for determining chromatin accessibility in frozen tissue homogenates using Benzonase, a robust nuclease whose efficacy is not affected by the presence of actin (Grøntved et. al, 2012). Here we describe a protocol to assess chromatin structure at specific loci using Benzonase digestion and qPCR of DNA extracted from frozen tissue homogenates and laser capture microdissected (LCM) defined cells. This protocol is relatively quick, inexpensive and achievable in any research laboratory outfitted with standard real time PCR equipment. This novel method will allow a more focused examination of chromatin structure, its influence on gene expression and the impact it may have on gene expression in normal and diseased brain as well as other tissues.

Disclosures: **E. Delvaux:** None. **D. Mastroeni:** None. **J. Nolz:** None. **P.D. Coleman:** None.

Poster

751. Molecular Techniques

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Himelic Family Foundation

Neuroscience Graduate Interdisciplinary Program at the University of Arizona

Beckman Foundation

Title: Translation Dysregulation in ALS

Authors: *S. YAO[1], A. COYNE[2], D. ZARNESCU[2];
[1]Mol. and Cell. Biol., [2]Univ. of Arizona, Tucson, AZ

Abstract: Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease that affects the lives of at least 30,000 people in the United States, annually. It is often categorized by the progressive neurodegeneration of motor neurons. TAR DNA Binding Protein (TDP-43) is normally found within the nucleus having known roles in RNA splicing as well as DNA-binding. Mislocalization of TDP-43 to the cytoplasm, either due to mutations or to environmental stressors leads to accumulation of TDP-43 in cytoplasmic inclusions. Notably, in the cytoplasm, TDP-43 associates with RNA stress granules and also affects mRNA translation, both of which are thought to contribute to disease pathology. In order to study the effects that cytoplasmic TDP-43 mutations have on translation, a technique called polysome fractionation is utilized. This technique uses sucrose gradients to effectively separate different ribosomal populations. The mRNAs that are bound to multiple ribosomes, or polysomes, are separated from the mRNAs bound to single ribosomes. This allows the pinpointing of specific defects in protein production as well as potential explanations to how it may affect cellular metabolism. Furthermore, this technique is able to provide information about the halting of protein production in response to stress which is a potential factor that contributes to ALS. Western blotting and quantitative PCR (qPCR) allow us to observe the protein and transcript distribution within the polyribosome fractions, respectively. Using these approaches we found that TDP-43 associates with both translating polyribosomes as well and untranslated fractions (RNP and the ribosomal subunits). qPCR of *futsch*, an mRNA target of TDP-43 showed a shift in transcript levels from actively translated to untranslated fractions in the context of TDP-43, indicating that *futsch* mRNA is being repressed by TDP-43 in motor neurons. Taken together, these findings indicate that TDP-43 regulates the translation of specific mRNAs and defects in translation may contribute to ALS. Experiments will focus on additional candidate mRNA targets that will be tested for their distribution within polysome fractionations to determine their translational status in disease. Candidate mRNAs that are identified by Translating Ribosome Affinity Purification (TRAP) that also associate with TDP-43 in complex will be tested. Specifically, qPCR will used to distinguish shifts in translational targets in polysomes. Targets that are downtranslated should exhibit a shift into the RNP fractions while uptranslated targets will exhibit a shift into the polysomes.

Disclosures: S. Yao: None. **A. Coyne:** None. **D. Zarnescu:** None.

Poster

751. Molecular Techniques

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Program#/Poster#: 751.24/MMM18

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: WT 103784MA

Title: Validation of the use of voltage-sensitive fluorescent proteins in *C. elegans*

Authors: *K. M. WEBSTER[1], M. CHATZIGEORGIOU[2], W. R. SCHAFER[1];
[1]MRC Lab. of Mol. Biol., Cambridge, United Kingdom; [2]Sars Intl. Ctr. for Marine Mol. Biol., Bergen, Norway

Abstract: *C. elegans* is an ideal model organism to use for studies involving optical imaging of neuronal activity. Its genetic tractability means that it is feasible to express genetically encoded proteins in specific neuronal populations, or even individual pairs of neurons. Many labs have successfully used genetically encoded calcium indicators (GECIs), such as Cameleon or GCaMPs, to monitor fluctuations in neuronal calcium levels in responses to a variety of stimuli. However, GECIs are regarded as a proxy for neuronal activity. It is technically challenging to record neuronal activity in *C. elegans* for several reasons, the foremost of which is that their pressurized cuticle makes accessing the neurons difficult. However, genetically encoded voltage-sensitive fluorescent proteins (VSFPs) offer an alternative to optically measure neuronal activity. The VSFP Butterfly 1.2 protein has been optimized to detect sub-threshold fluctuations in neural activity in mammalian cells. We have been able to visualize and record activity from a codon optimized version of VSFP Butterfly 1.2 in *C. elegans* neurons. This construct successfully traffics to the plasma membrane and is functional in multiple types of neurons. We have expressed this protein under a variety of promoters and have used it to record neuronal responses to mechanical and other stimuli, including nose press. It is also possible to record spontaneous activity from motor neurons in young, freely moving worms. The combination of the stereotyped development of the *C. elegans* nervous system with the known physical connectome and well characterized functional circuits presents the opportunity to use this tool to simultaneously record the activity of an entire circuit. Further, we are able to correlate the changes in fluorescence intensity in the two different components of the FRET pair with specific voltage changes across the membrane in a whole cell patch-clamp electrophysiology configuration in dissociated *C. elegans* neurons. The data we present validate the usefulness of VSFP Butterfly 1.2 as a method to optically measure neuronal activity in *C. elegans* and demonstrate the range of experimental uses to which it can be applied.

Disclosures: **K.M. Webster:** None. **M. Chatzigeorgiou:** None. **W.R. Schafer:** None.

Poster

751. Molecular Techniques

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Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 751.25/MMM19

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: Enrichment of unamplified DNA and long-read smrt sequencing to unlock repeat expansion disorders

Authors: *J. EKHOLM[1], Y.-C. TSAI[2], T. A. CLARK[2];
[1]Marketing, Pacific Biosci. Inc, Menlo Park, CA; [2]Pacific Biosci., Menlo Park, CA

Abstract: Many neurological and neuromuscular disorders are caused by repeat expansions that span large genomic regions. Despite the fact that the causative disease genes have been uncovered, the underlying biological disease mechanisms are still largely unknown. This is mainly due to technological limitations that do not allow for the needed base-pair resolution of the repetitive DNA. Research has shown that in addition to accurately accessing the repetitive elements, the interruption sequences found in the midst of these repetitive regions have an impact on DNA stability, disease anticipation and disease severity. Other aspects that seem to be key in further understanding the disease etiology of repeat expansion disorders are base modifications and the transcriptional activity of the mutated genes.
We have developed a novel, amplification-free enrichment technique that uses the CRISPR/Cas9 system to target large repeat expansions. This method, in conjunction with PacBio's long reads and uniform coverage, enables sequencing of these complex genomic regions. By using a PCR-free amplification method, we are able to access not only the repetitive elements and interruption sequences accurately, but also the epigenetic information. In addition, we also avoid any biases that would be introduced by PCR. This method has successfully been used in sequencing the causative repeat expansions for Huntington's Disease (HTT; CAG repeat), Fragile X (FMR1; CGG repeat), ALS (C9orf72; GGGGCC repeat), and Spinocerebellar ataxia type 10 (SCA10; variable ATTCT repeat). With this data, we demonstrate the ability to isolate hundreds of individual on-target molecules and accurately sequence through long repeat stretches, regardless of the extreme GC-content. We also demonstrate the ability to directly detect and characterize epigenetic signatures. Lastly, we have also built the first comprehensive isoform map for the FMR1 gene and identified transcripts that seem to have functional relevance in the pathology of FMR1-associated disorders such as fragile X-associated tremor/ataxia syndrome.

Disclosures: **J. Ekholm:** A. Employment/Salary (full or part-time): Pacific Biosciences. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pacific Biosciences. **Y. Tsai:** A. Employment/Salary (full or part-time): Pacific Biosciences. E. Ownership Interest (stock, stock

options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pacific Biosciences. **T.A. Clark:** A. Employment/Salary (full or part-time): Pacific Biosciences. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pacific Biosciences.

Poster

751. Molecular Techniques

Location: Halls B-H

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Program#/Poster#: 751.26/MMM20

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: TargetBraIn (HEALTH-F2-2012-279017)

BrainPath (PIAPP-GA-2013-612360)

DFG (AS-464/1-1)

Title: Two-color bioluminescence for sensitive and quantitative discrimination of cell grafts in the mouse brain

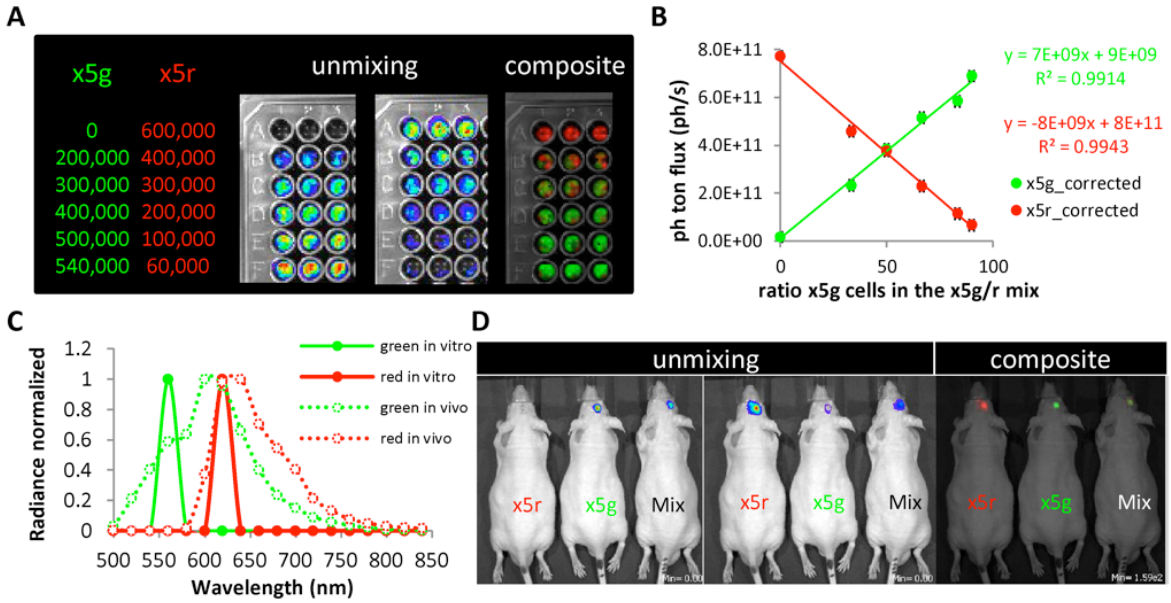
Authors: *M. ASWENDT[1], S. VOGEL[1], C. SCHÄFER[1], A. JATHOUL[2], M. HOEHN[1,3]; [1]Max Planck Inst. For Metabolism Res., Koeln, Germany; [2]Sch. of Biosci., Cardiff Univ., Cardiff, United Kingdom; [3]Radiology, Leiden Univ. Med. Ctr., Leiden, Netherlands

Abstract: Objectives
Bioluminescence imaging (BLI) holds great potential for sensitive and quantitative in vivo monitoring of mouse brain cells. We have validated and optimized BLI for tracking viability and fate of transplanted stem cells longitudinally. Here, we present a novel multicolor luciferase approach, in which spectral unmixing is applied to calculate individual cell numbers in vivo.
Methods
We used firefly luciferases x5g and x5r, which are codon-optimized for high expression in mammalian cells and mutated for green (Em=540 nm) and red (Em=614 nm) photon emission. Luciferases were cloned into lentiviral plasmids and used to transfect HEK-293T cells. BLI and spectral unmixing was performed using the IVIS Spectrum CT system (18 emission filter, 500-840 nm). A cell dilution series with variable ratio of x5g/r cells was used *in vitro* (1 mM D-Luciferin, 1 min acquisition). Discrimination and quantification by imaging cell grafts containing different mixtures of x5r and x5g cells (50,000-150,000 cells) in the cortex or striatum of nude (Nu/Nu) mice (n=40) *in vivo* (300 mg/kg D-Luciferin i.p. pre-Isoflurane anaesthesia).
Results

The spectral unmixing algorithm was found to predict the ratio of x5g/r cells in the micture accurately because of the strictly linear relation between photon flux and x5g/r cell numbers (Fig. 1A, B). The in vivo spectra of x5g/r were shifted towards higher wavelengths and much broader, however, still distinct enough to be identified as two emission peaks by the spectral unmixing algorithm (Fig. 1 C). The in vivo data on cortical and striatal cell grafts revealed efficient spectral unmixing of red and green signals in correct quantity (e.g. 71% x5g calculated by the algorithm and 75% x5g actually transplanted) (Fig. 1 D).

Conclusions

We have demonstrated efficient unmixing of novel red and green firefly luciferases *in vitro* and *in vivo*. For the first time, this two-color BLI promotes sensitive and quantitative tracking of distinct cell populations. We are currently translating this approach to the spectral unmixing of neurons and astrocytes in vivo.



Disclosures: **M. Aswendt:** None. **S. Vogel:** None. **C. Schäfer:** None. **A. Jathoul:** None. **M. Hoehn:** None.

Poster

752. Cellular and Network Electrophysiological Approaches

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Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

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Topic: I.04. Physiological Methods

Support: The smart IT convergence system research center funded by the ministry of education, science and technology as global frontier project(CISS-2012M3A6A6054204)

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Title: Analysis on neurotoxicity of gold nanoparticles on neurons and glia

Authors: *S. KIM[1], S. HWANG[1], S. JUN[1,2];
[1]Electronics Engin., [2]Dept. of Brain and Cognitive Sci., Ewha Womans Univ., Seoul, Korea, Republic of

Abstract: Among various types of nanoparticles, gold nanoparticles in particular are stealing the spotlight for its stability and suitability for bio-related studies. Recently, for various purposes such as bioimaging, drug delivery, and neuromodulation, researches utilizing nanoparticles are being conducted. Whereas gold nanoparticles are known to be biocompatible, the particle itself is, after all, a foreign substance both *in vivo* and *in vitro*. To date, there have been prior investigations to assess the toxicity of nanoparticles upon non-neuronal cells. In this study, we attempt to assess neurotoxicity of gold nanoparticles by observing cultured hippocampal neurons and glial cells. They were separately cultured and grown for 14 days, seeded with gold nanorods, and then observed for seven consecutive days. The particles used were 59 nm and 146 nm in length, and the concentration rate varies from $8.5x10^{10}$ nps/mL to $3.4x10^{11}$ nps/mL. The effects are different depending on the size and the concentration of the particles. We focus especially on the aspect of cell death linked with neurotoxicity, mainly utilizing live dead cell assays. In addition, correlation between glial death and the survival of neurons under nanoparticle-seeded environment are interpreted. In general, severe toxicity was observed with the larger size and heavier concentration rates. This study is expected to serve as a foundation experiment for further research on the cytotoxicity of gold nanoparticles. Furthermore, *in vivo* experiments by injecting gold nanoparticles in Sprague-Dawley rats are to follow.

Disclosures: **S. Kim:** None. **S. Hwang:** None. **S. Jun:** None.

Poster

752. Cellular and Network Electrophysiological Approaches

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 752.02/MMM22

Topic: I.04. Physiological Methods

Support: NIH Brain Initiative Grant (1U01MH106027-015)

Title: Low access resistance subcortical whole cell recordings *In vivo*

Authors: *W. STOY[1], Y. J. LIEW[1], B. YANG[2], C. J. WHITMIRE[1], A. PALA[1], C. CAPOCASALE[2], T. LEE[2], A. ORTIZ[1], P. Y. BORDEN[1], G. B. STANLEY[1], C. R. FOREST[2,1]; [1]Wallace H. Coulter Dept. of Biomed. Engin., [2]George W. Woodruff Sch. of Mechanical Engin., Georgia Inst. of Technol., Atlanta, GA

Abstract: Patch clamp recordings deep in the intact living brain (e.g. >1 mm) have long suffered from low yield and high access resistance, primarily due to pipette electrode contamination during descent to subcortical nuclei. In the mouse, for example, pipettes are significantly less likely to be inserted cleanly to the thalamus, located at a depth of 3 mm in C57BL/6 mice as compared to, the cortex, located at a depth of approximately 0.5 mm. This degradation limits the utility of patch clamping, the gold-standard method of cellular electrophysiology, where it could be extremely useful for studying cellular function and synaptic connectivity below superficial layers. In addition, this limitation renders patch clamping virtually prohibitive for large brain models such as Rhesus macaque.

After systematically measuring the effect of depth on pipette cleanliness, we developed an algorithm and best practices for reducing pipette tip contamination and improving access resistance when targeting deep, subcortical neurons in mice. Our results show that decent to depth of 0.5 mm increases electrode resistance by more than 300 kOhm in 32% of trials, while descents to 3.0 mm increase electrode resistance above this threshold in 88% of trials.

Our algorithm and methods improve these results substantially. By utilizing robotically controlled pipette motion and continuous electrical resistance feedback, we are able to "dodge" obstructions such as neurons and blood vessels during descent. Using this new algorithm, pipette resistance is measured at 128 Hz during descent for a high spatial resolution. If an obstruction is encountered prior to the region of interest (as indicated by an increase in resistance >1 MOhm threshold), the pipette is stopped, retracted, and moved laterally in 20 um steps. Lateral steps are repeated until resistance decreases below threshold, indicating that the obstruction has been circumvented and descent is resumed until the region of interest is reached.

Pipettes inserted using this method arrived at the region of interest without a significant change in resistance (<300 kOhms at 3 mm) 69% of the time (n=46), while pipettes inserted using traditional blind, linear methods were inserted successfully 32% of the time (n=31); Whole cell recordings following successful descent resulted in lower access resistance than those inserted with the traditional algorithm. This pipette localization method therefore greatly improves the quality and accessibility of subcortical tissues in the intact brain.

Disclosures: W. Stoy: None. **Y.J. Liew:** None. **B. Yang:** None. **C.J. Whitmire:** None. **A. Pala:** None. **C. Capocasale:** None. **T. Lee:** None. **A. Ortiz:** None. **P.Y. Borden:** None. **G.B. Stanley:** None. **C.R. Forest:** None.

Poster

752. Cellular and Network Electrophysiological Approaches

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 752.03/MMM23

Topic: I.04. Physiological Methods

Support: NIH Brain Initiative Grant (1U01MH106027-015)

Title: Rapid cortical barrel mapping using automated multi-whisker stimulation and intrinsic optical signal imaging

Authors: *T. LEE[1], C. M. CAPOCASALE[1], P. Y. BORDEN[2], W. STOY[2], C. J. WHITMIRE[2], Y. LIEW[2], A. PALA[2], A. D. ORTIZ[2], B. YANG[1], G. B. STANLEY[2], C. R. FOREST[1];
[1]George W. Woodruff Sch. of Mechanical Engin., [2]Wallace H. Coulter Dept. of Biomed. Engin., Georgia Inst. of Technol., Atlanta, GA

Abstract: The study of single-unit, whisker-evoked activity in the mouse somatosensory cortex through targeted electrophysiology requires multi-whisker stimulation and knowledge of the corresponding barrel location. Tools for addressing multiple whiskers are slow and manual (e.g. galvanometer) or inordinately expensive and complex (e.g. piezoelectric array). Imaging tools for barrel mapping have been well studied but require users to manually relate manipulator coordinates to anatomical coordinates. To address these limitations, we describe two tools for rapid, automated cortical barrel mapping: (1) a low cost, compact device for multi-whisker probing and (2) an automated system for Intrinsic Optical Signal Imaging (IOSI) barrel localization and precision electrode placement. (1) To provide independent actuation of vibrissa, whiskers are manually threaded into a comb-like, linear array. Individual whiskers are constrained in the dorsal-ventral direction but free to move in the rostral-caudal direction. Whiskers are actuated within the slot of the comb-like array in the rostral-caudal direction using directed puffs of air; we can deliver a maximum angular velocity of 250 degrees per second and a maximum absolute deflection of 5 degrees at the whisker pad (stimulator located 10 mm from the facial skin) at up to 10 Hz. To functionally validate the stimulator, we used voltage sensitive fluorescent protein imaging of barrel activation in response to whisker movement from our stimulator and from a galvanometer. We find no difference in the cortical barrel centroid location and the spatial spread of barrel activation between the two stimulating paradigms. (2) In conjunction with the whisker stimulator, we have developed a system for automated, IOSI-guided, electrode placement in the barrel cortex. Prior to cortical optical imaging, the electrode tip is imaged and located in the imaging volume. Cortical barrel centroids are found immediately using IOSI followed by automated image processing for rapid functional mapping. We report accurate and repeatable IOSI-guided, automated barrel localization when compared against post-mortem histological stains. With machine vision, we transform electrode coordinates into

manipulator coordinates for precision electrode placement. We have demonstrated the ability to automatically place an electrode at desired positions within the system's imaging volume to within 40.3 μm, 95% of the time. Integrating these tools enables rapid, automated barrel mapping and targeted electrode placement.

Disclosures: **T. Lee:** None. **C.M. Capocasale:** None. **P.Y. Borden:** None. **W. Stoy:** None. **C.J. Whitmire:** None. **Y. Liew:** None. **A. Pala:** None. **A.D. Ortiz:** None. **B. Yang:** None. **G.B. Stanley:** None. **C.R. Forest:** None.

Poster

752. Cellular and Network Electrophysiological Approaches

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Topic: I.04. Physiological Methods

Support: NIH Brain Initiative Grant (1U01MH106027-015)

Title: Experimental and analytical approaches for multi-site electrophysiology in the topographically aligned thalamocortical circuit

Authors: ***Y. LIEW**[1], C. J. WHITMIRE[1], W. A. STOY[1], A. PALA[1], A. SEDERBERG[1], A. D. ORTIZ[1], P. Y. BORDEN[1], B. YANG[2], C. M. CAPOCASALE[2], T. LEE[2], C. R. FOREST[2], G. B. STANLEY[1];
[1]Wallace H Coulter Dept. of Biomed. Engin., [2]George W Woodruff Sch. of Mechanical Engin., Georgia Inst. of Technol., Atlanta, GA

Abstract: Dynamic interaction between neurons determines the nature and efficiency of information transmission across neural networks. To quantify sensory encoding at each stage of neural processing, simultaneous recording along a feedforward pathway is critical yet challenging. Previous work has demonstrated that targeting topographically aligned regions in vivo is experimentally tractable and could reveal synaptically coupled neurons in the intact circuit. However, a well-documented systematic approach is lacking. In this work, we optimize the experimental approach to reliably record from neuronal pairs in the thalamocortical (TC) circuit of the vibrissa pathway in the anesthetized rodent and assess the conventional spike cross-correlation methods typically used to infer monosynaptic connectivity from simultaneously recorded neurons. Taking advantage of the well-known anatomical and functional organization of the rodent's whisker pathway, we first perform functional mapping of primary somatosensory cortex (S1). We assess the single unit layer 4 response to deflections of different individual whiskers, and create a stereotypical functional map to align with a template anatomical map

using stereotaxic coordinates. After identifying a barreloid in the ventroposteromedial thalamus (Vpm) responding to one primary whisker, we then utilize the map to target the corresponding cortical barrel. To verify the accuracy of the thalamic and cortical recording locations, we quantify response latencies and adapting properties of the neuronal pair in response to whisker deflection. Drawing inference of monosynaptic connection relies on spike count of coordinated firing between thalamic and cortical neurons, which is challenging given the typically low spontaneous TC activity. To probe the TC synaptic connectivity, we deliver a low velocity sinusoidal whisker stimulus to evoke decorrelated thalamic spikes while performing simultaneous recordings in topographically aligned TC regions. Using cross-correlation analysis of the spiking activity, we examine the functional relationship between identified neuronal pairs and classify monosynaptic connectivity. Systematic evaluation of the effects of parameters such as length of acquired data and the absolute firing rate of the recorded neurons suggests a set of guidelines for assessing monosynaptic connectivity through this method. Taken together, we have developed a step-by-step experimental and analytical approach for obtaining paired neural data, laying the foundation for multi-site, multi-electrode and even intracellular recording across different connected brain structures.

Disclosures: **Y. Liew:** None. **C.J. Whitmire:** None. **W.A. Stoy:** None. **A. Pala:** None. **A. Sederberg:** None. **A.D. Ortiz:** None. **P.Y. Borden:** None. **B. Yang:** None. **C.M. Capocasale:** None. **T. Lee:** None. **C.R. Forest:** None. **G.B. Stanley:** None.

Poster

752. Cellular and Network Electrophysiological Approaches

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Program#/Poster#: 752.05/MMM25

Topic: I.04. Physiological Methods

Support: 1U01MH106027-015 (IK, WAS, CRF)

NIH (NINDS) NS089719 (AJ)

T32 GM008602 (OAM)

Title: Cleaning patch-clamp pipettes enables their re-use

Authors: ***I. KOLB**[1], W. A. STOY[1], E. ROUSSEAU[2], O. A. MOODY[3], A. JENKINS[4], C. R. FOREST[1];
[1]Georgia Inst. of Technol., Atlanta, GA; [2]SUNY Polytechnic Inst., Albany, NY; [3]Emory Univ., Atlanta, GA; [4]Emory Univ. Sch. of Med., Atlanta, GA

Abstract: Patch-clamp recording is a gold-standard technique for the accurate measurement of membrane voltage and current fluctuations in electrically active cells. From the advent of the technique in the late 1970s, it has been widely accepted that using a fresh pipette to patch every cell is critical to form a high-quality gigaohm-seal with the cell and yield a successful recording. On the other hand, the ability to reuse pipettes would greatly facilitate patch-clamp experiments, particularly those involving specially-treated pipette tips or multiple simultaneous recordings. We demonstrate for the first time that a simple cleaning procedure can enable pipettes to be reused. This fast (60 s) fully-automated cleaning procedure consists of dipping used pipettes into a commercially available detergent, pneumatically forcing the detergent into the tip, and finally rinsing the tip in a non-cytotoxic solution.

With pipette cleaning, 8 pipettes were used to whole-cell patch 84 Human Embryonic Kidney (HEK293) cells out of 88 attempts (95% success rate), demonstrating that pipettes can be reused 10 times. To confirm, we patched and reused pipettes on neurons in culture, acute brain slice, and in-vivo (Fig. 1). The pharmacological safety of pipette cleaning was demonstrated by patching HEK293 cells transfected with a γ-Aminobutyric acid receptor type A ($GABA_AR$). No difference was seen in the dose response of cells patched with fresh and cleaned pipettes in response to GABA (peak current: fresh: 3.1 ± 1.54 nA, n=13, after 4^{th} clean: 2.8 ± 1.13 nA, n=8). Finally, we integrated the pipette cleaning algorithm into our previously-developed automated patch-clamp robot, the Autopatcher. This enabled the Autopatcher to patch multiple HEK293 cells serially, without human intervention, at a maximal rate of approximately 1 cell per 5 min. These results demonstrate for the first time that pipettes can be reused multiple times with a simple automated cleaning procedure, yielding a widely applicable tool for a variety of patch-clamp preparations, especially those requiring high throughput and automation.

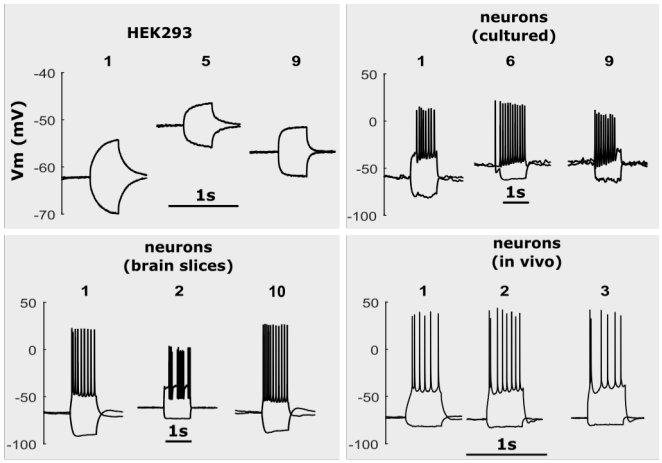

Figure 1: A single pipette can be used to patch-clamp multiple cells in different experimental preparations. Number above recording indicates number of times pipette was used.

Disclosures: I. Kolb: None. **W.A. Stoy:** None. **E. Rousseau:** None. **O.A. Moody:** None. **A. Jenkins:** None. **C.R. Forest:** None.

Poster

752. Cellular and Network Electrophysiological Approaches

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Program#/Poster#: 752.06/MMM26

Topic: I.04. Physiological Methods

Support: Paul G. Allen

Title: Scaled electrophysiological, morphological, and transcriptomic characterization of mouse cortical neurons using an *In vitro* slice electrophysiology platform.

Authors: K. HADLEY, B. LEE, T. KIM, D. HILL, T. JARSKY, L. KIM, R. MANN, A. OLDRE, S. SORENSEN, K. SMITH, *J. BERG, B. TASIC, G. MURPHY, H. ZENG;
Allen Inst. For Brain Sci., Seattle, WA

Abstract: The Allen Institute has undertaken a comprehensive, multi-year endeavor to understand the diversity and hierarchical organization of neuronal cell types. As part of this effort, the Institute has published electrophysiological, morphological, and transcriptomic data from hundreds of individual neurons of the mouse brain in the Allen Cell Types Database. The existing program utilizes parallel pathways for transcriptomic and electrophysiological/morphologic profiling, both of which take advantage of Cre lines that target subpopulations of neurons and allow for the comparison of the same types of cells across many animals: an *in vitro* slice electrophysiology platform allows for concurrent cell recording and filling with biocytin, followed by histological processing, imaging, and morphological reconstruction, while RNA-Seq is performed on dissociated single cells isolated by fluorescence activated cell sorting (FACS). In order to combine these two approaches and collect data from all three modalities for each individual cell, we have developed an approach to obtain RNA sequence data from recorded cells while maintaining intact morphologies from a substantial fraction of neurons. To scale this technique to reliably obtain high quality transcriptome and morphology data across multiple electrophysiology rigs and operators, we have standardized specific aspects of the protocol. Patch pipette characteristics, contents of the internal solution, and pressures required for cytoplasmic extraction, among other features, have been optimized to maximize the amount of electrophysiology data generated, while increasing the quality of transcriptomic data, as well as the morphological integrity. The ability to collect and analyze all three types of data from a single neuron will further our ongoing efforts to identify distinct cell classes and expand our understanding of the genetic mechanisms underlying neuronal diversity in the cortex.

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Poster

752. Cellular and Network Electrophysiological Approaches

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Program#/Poster#: 752.07/MMM27

Topic: I.04. Physiological Methods

Title: *In vitro* electrophysiological recordings of multiple brain regions from aged pig tailed Macaques

Authors: A. D. WHYMENT[1,2], F. ZHAO[1,2], M. VAN DEN TOP[1,2], J. PRYOR[1,3], *D. SPANSWICK[1,3,2];
[1]NeuroSolutions Ltd., Coventry, United Kingdom; [2]Pacific Discovery Services, Melbourne, Australia; [3]Dept. of Physiol., Monash Univ., Melbourne, Australia

Abstract: The relatively long lifespan, extended infancy and social cognition of non-human primates (NHPs) parallels many aspects of human development and the physiological, behavioural, and neuroanatomical similarities to humans can facilitate translation of therapeutic strategies to a range of human conditions. To date, the vast majority of electrophysiological recordings performed in NHPs have been achieved in juvenile animals with little if any work performed in aged animals. Here, data was obtained from two aged (20 years) female pig-tailed macaques (Macaque nemestrina). Both animals were scheduled for euthanasia due to significant health issues. On the day of experiments, animals were maintained under deep anaesthesia and a craniotomy performed to expose the brain. Animals were perfused with ice-cold, oxygenated artificial cerebrospinal fluid (aCSF, in mM: NaCl 124, KCl 5, KH_2PO_4 3, $MgCl_2$ 2, $CaCl_2$ 2, $NaHCO_3$ 23 and glucose 10) to displace blood and to rapidly reduce the temperature of the brain. The brain was removed within a few minutes of death and specific regions were immediately dissected for subsequent slice preparation and *in vitro* electrophysiological recording. The post-mortem interval between death and tissue acquisition was measured in minutes as compared to the often hours to days that can elapse in other studies. Brain slices of 400 μm thickness were cut in chilled (<4°C) aCSF using multiple Leica VT1000s microtomes. Whole-cell patch-clamp recordings were successfully performed from multiple brain regions from a single animal with recordings performed from neurones within slices prepared from cortical, hippocampal, striatal, hypothalamic and cerebellar tissue. Simultaneously, extracellular recordings were performed in hippocampal slice tissue and long-term potentiation (LTP) of field excitatory postsynaptic potentials (fEPSPs) induced in the CA1 region following brief high-frequency electrical stimulation of the Schafer-collateral pathway. This approach enabled *in vitro* brain slice preparations to be maintained viable for at least 36 hours with the intrinsic, electrophysiological, synaptic and pharmacological properties of neurones characterised over this period. Our data show the viability of recording *in vitro* in aged NHP and highlights the potential of such studies

for future translational studies, maximising the opportunity for NHP tissue use whilst minimising animal suffering.

Disclosures: **A.D. Whyment:** A. Employment/Salary (full or part-time): NeuroSolutions Ltd. **F. Zhao:** A. Employment/Salary (full or part-time): NeuroSolutions Ltd. **M. van den Top:** A. Employment/Salary (full or part-time): NeuroSolutions Ltd. **J. Pryor:** A. Employment/Salary (full or part-time): NeuroSolutions Ltd. **D. Spanswick:** A. Employment/Salary (full or part-time): NeuroSolutions Ltd.

Poster

752. Cellular and Network Electrophysiological Approaches

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Topic: I.04. Physiological Methods

Support: NIH/NIBIB R01 EB016101

NIH/NINDS F32 NS093897

Title: Flexible microelectrode arrays for high-density subsecond dopamine recording

Authors: ***H. N. SCHWERDT**[1,2], M. KIM[2], D. HOMMA[2], S. AMEMORI[2], T. YOSHIDA[2], H. SHIMAZU[2], R. S. LANGER[1], A. M. GRAYBIEL[2], M. J. CIMA[1];
[1]Koch Inst. for Integrative Cancer Res., [2]McGovern Inst. for Brain Res., MIT, Cambridge, MA

Abstract: Multichannel electrochemical recording techniques are needed to reliably monitor the spatiotemporal activity of dopamine (DA) and other electroactive neurotransmitters, which collectively influence brain function. Fast-scan cyclic voltammetry (FSCV) is a well-established technique for monitoring changes in DA concentration and other electroactive substances with *millisecond* temporal resolution. Experiments using FSCV have shown that the spatiotemporal dynamics of DA release are heterogeneous within the striatum, a region that receives robust DA innervation. Traditional carbon fiber (CF) microelectrodes used in FSCV are, however, usually encased in glass or fused silica shafts with nominal diameters of ~100 μm that may compromise the intrinsic functionality and vasculature of the brain sites into which they are introduced. Persisting inflammatory responses to these implants may further alter intrinsic DA signaling processes as well as those recorded by such sensors. We have developed flexible CF microelectrode arrays (MEAs) in order to enhance electrochemical recording functionality and to ameliorate chronic inflammatory processes. Microfabrication processes are used to construct 8-channel CF MEAs having individual electrode lengths of 5-6 mm with a pitch of 250 μm. CF

electrodes are insulated with ~700 nm thick parylene-C for overall diameters of ~8.4 μm. Active recording sites are formed by exposing a ~150 μm length from the CF tips. DA recording capacity was assessed *in vitro* and *in vivo* in anesthetized rats. DA concentration was recorded concurrently from 4 recording sites of the CF MEA across the mediolateral axis of the dorsal striatum as evoked by medial forebrain bundle (MFB) stimulation. Controlled current stimulation was delivered at bipolar platinum-iridium electrodes with a train of 48 biphasic pulses (2 ms pulse-width, 60 Hz, 200 μA). DA selectivity was conferred by demonstration of redox potentials at ~−0.25 and 0.6 V and by principle component analysis. The concentration of DA induced across the 4 sites was heterogeneous and dependent on location of MFB electrodes, with evoked DA oxidation current amplitudes ranging from −5 to 40 nA. Our results demonstrate the potential of microfabricated CF MEAs in elaborating the spatiotemporal dynamics of DA and other neurotransmitters in the brain.

Disclosures: H.N. Schwerdt: None. **M. Kim:** None. **D. Homma:** None. **S. Amemori:** None. **T. Yoshida:** None. **H. Shimazu:** None. **R.S. Langer:** None. **A.M. Graybiel:** None. **M.J. Cima:** None.

Poster

752. Cellular and Network Electrophysiological Approaches

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Topic: I.04. Physiological Methods

Support: DARPA Young Faculty Award

Hamill Foundation

Title: Nano-electrode arrays for simultaneous optical and electrical interrogation of *C. elegans* and Hydra

Authors: *K. N. BADHIWALA[1], D. L. GONZALES[2,3], D. G. VERCOSA[2,3], C. DUPRE[4], R. YUSTE[4], J. T. ROBINSON[1,2,3,5];
[1]Bioengineering, [2]Applied Physics Program, [3]Electrical and Computer Engin., Rice Univ., Houston, TX; [4]Biol. Sci. and Neurosci., Columbia Univ., New York, NY; [5]Neurosci., Baylor Col. of Med., Houston, TX

Abstract: Advances in technologies for electrophysiology combined with optical and genetic manipulations have greatly improved our understanding of the brain. Yet, the complexity of the human brain makes it difficult to develop a complete understanding of how sensory information

is processed and transformed into behavior, and how this information processing changes with time. To better understand the dynamics of an entire nervous system, it is useful to work with model organisms with fewer neurons. Small transparent organisms like the roundworm *C. elegans* and freshwater jellyfish *Hydra vulgaris* provide us with nervous systems that can be imaged in their entirety at cellular resolution using a variety of optical microscopy techniques. While these optical methods provide excellent spatial resolution, they typically lack the millisecond temporal resolution possible with electrophysiology. Thus electrophysiology is an important complement to optical techniques that would provide precise timing of individual action potentials. However, electrophysiology remains a difficult and invasive process in these small organisms. Here we present a platform technology that can perform simultaneous high-resolution optical imaging and electrophysiology in small model organisms. This device is based on our recently developed nanoscale suspended electrode arrays (nano-SPEARS) combined with inverted confocal microscopy allowing us to image neuronal calcium activity while simultaneously measuring electrical activity associated with rhythmic potentials in *Hydra* or neuromuscular junction activity in worms. Taking advantage of the microfluidic environment, we can also measure the response of organisms to specific stimuli such as chemical or thermal cues. As a result, we can improve the understanding of neural information processing by probing whole-brain neural dynamics with high-temporal, and high-spatial resolution in small model organisms as they interact with their environment.

Disclosures: **K.N. Badhiwala:** None. **D.L. Gonzales:** None. **D.G. Vercosa:** None. **C. Dupre:** None. **R. Yuste:** None. **J.T. Robinson:** None.

Poster

752. Cellular and Network Electrophysiological Approaches

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Program#/Poster#: 752.10/MMM30

Topic: I.04. Physiological Methods

Support: NSF-RISE Grant (HRD1345215)

Title: Nano electrode array for electrical impedance tomography of neural network activity.

Authors: *H. YOON, M. H. KIM;
Norfolk State Univ., Yorktown, VA

Abstract: Real time imaging of fast electrical activity in the nervous system is a major current goal in neuroscience. Electrical Impedance Tomography (EIT) enables tomographic imaging of impedance changes related to fast neuronal activity. For neural activity imaging, EIT has been

implemented with surface electrodes placed on the skull or directly on the surface of the cerebral cortex in recent researches. The aim of this research is to perform EIT in the deep brain using depth electrode arrays. As part of an effort to develop deep electrode array, we applied nanotechnology on electrodes to enhance electrochemical impedance characteristics. In this research, various designs of depth probes were investigated to minimize damage to the brain tissue and overcome size constraints in the brain. For nano-electrode fabrication, we applied electrodeposited iridium oxide film neural probes. Iridium oxide showed high charge density and high charge injection rate which are ideal for EIT application. To enhance mechanical adhesion of the nanostructure film, we developed a new fabrication process that amplifies the nucleation of iridium oxide at the initial stage of deposition process. Measured results were analyzed using electrical impedance spectroscopy and compared with theoretical models. Measured electrochemical impedance values demonstrated significant reduction of electrochemical impedance. Applying measured impedance values and the size of electrodes, a spatial resolution of EIT imaging is expected to be less than 200 μm. Future work is required to apply nano-depth probes for in-vitro and in-vivo studies and characterize their functionality in the rat brain model.

Disclosures: H. Yoon: None. **M.H. Kim:** None.

Poster

752. Cellular and Network Electrophysiological Approaches

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 752.11/MMM31

Topic: I.04. Physiological Methods

Title: MIES: a electrophysiology experiment automation software.

Authors: *T. JARSKY[1], L. CAMPAGNOLA[1], D. REID[1], J. BERG[1], T. BRAUN[2];
[1]Allen Inst. for Brain Sci., Seattle, WA; [2]() byte physics, Berlin, Germany

Abstract: Multi-channel Igor Electrophysiology Software (MIES) is a major component of the software tools the Allen Institute for Brain Science is using to fully automate electrophysiology recordings. A key feature of MIES, not commonly found in other electrophysiology data acquisition software, is the ability to acquire data on more than four channels at the same time. To counteract the complexity that arises from recordings from multiple cells simultaneously, MIES provides an integrated user interface that simplifies hardware control and data visualization. Another mechanism by which MIES facilitates data acquisition on multiple channels is by automation of experiment documentation. Finally, MIES automates pressure regulation, with the use of only two commercial off-the-shelf components, to relieve the user of this manually intensive task. MIES is built with the widely used, affordable, well supported, and

professionally documented scientific and engineering software package Igor Pro (Wavemetrics), however, MIES data is accessible outside of Igor because data is stored the standardized data format Neurodata Without Borders (NWB). MIES is designed to integrate with other software packages for imaging or micromanipulator automation. MIES has been comprehensively tested, in part, because of its primary role in data acquisition for the Allen Cell Types Database. In short, MIES is scalable, comprehensive, and customizable and is intended for acquisition, visualization, and analysis of electrophysiology data on multiple channels simultaneously.

Disclosures: **T. Jarsky:** None. **L. Campagnola:** None. **D. Reid:** None. **J. Berg:** None. **T. Braun:** None.

Poster

752. Cellular and Network Electrophysiological Approaches

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Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 752.12/MMM32

Topic: I.04. Physiological Methods

Title: Microfluidic actuation of flexible microelectrodes for neural recording and stimulation

Authors: *F. VITALE[1], D. G. VERCOSA[2], S. PAMULAPATI[3], J. YAN[4], K. BADHIWALA[4], M. PASQUALI[3], J. T. ROBINSON[5];
[1]Ctr. for Neuroengineering and Therapeut., Univ. of Pennsylvania, Philadelphia, PA; [2]Applied Physics, [3]Chem. and Biomolecular Engin., [4]Bioengineering, [5]Electrical and Computer Engin., Rice Univ., Houston, TX

Abstract: The development of implantable devices to record and stimulate neural circuits has led to breakthrough discoveries on the connectivity and functionality of the brain in healthy and diseased states. Despite tremendous advances, technologies for high-resolution electrical recording and modulation of neural activity at the cellular level still rely on rigid metals or silicon components, which poorly match soft brain tissue and cause extensive acute and chronic injury.[1,2]
Flexible electrodes and ultra-small microwires approximating cellular scale have been shown to significantly reduce neural damage during chronic implantation and increase the quality and longevity of neural recordings.[3] However, flexible electrodes require stiffening agents (i.e., polymeric coatings or rigid needles) to overcome the buckling force upon implantation. These stiffening agents increase device footprint and potentially cause additional neuronal death or damage to the blood brain barrier during implantation.[3]
To overcome this challenge we have developed a novel technology to precisely actuate, control and implant high-performance, soft carbon nanotube fiber (CNTf) microelectrodes without using

a stiffening agent or shuttle. Instead, our technology uses fluid flow within a microfluidic device to drive electrodes into tissue. The hydraulic design of the microfluidic device and the on-chip valves enable precise bidirectional control of the fiber motion, velocity and position, by simply tuning the flow parameters. *In vitro* experiments in agar phantoms mimicking the mechanical properties of the brain, show that microfluidic actuated CNTf can be implanted at 1.5 mm depth with 30 μm precision, while keeping the total volume of fluid injected below 0.1 μL.
To demonstrate the feasibility of this novel technology as a tool for inserting flexible CNTf electrodes and probing neural activity *in vivo,* we utilized the small cnidarian *Hydra vulgaris* as model organism. CNTfs were implanted in *Hydra* and used to interrogate neural circuits over a period of several hours and isolate the specific activity of the neural networks located in different regions of the *Hydra* body.
1. Subbaroyan, J; Martin, D. C; Kipke, D. R. *J Neural Eng* **2005**, *2* (4), 103–113.
2. Harris, J. P; Tyler, D. J. *Crit Rev Biomed Eng* **2013**, *41* (6), 435–456.
3. Vitale, F; Summerson, S. R; Aazhang, B; Kemere, C; Pasquali, M. *ACS Nano* **2015**, *9* (4), 4465–4474.
F.V. and D.G.V. contributed equally to this work

Disclosures: **F. Vitale:** None. **D.G. Vercosa:** None. **S. Pamulapati:** None. **J. Yan:** None. **K. Badhiwala:** None. **M. Pasquali:** None. **J.T. Robinson:** None.

Poster

752. Cellular and Network Electrophysiological Approaches

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Topic: I.04. Physiological Methods

Support: Samsung Fellowship

NIH 1R01EY023173

NIH Director's Pioneer Award 1DP1NS087724

New York Stem Cell Foundation-Robertson Award

Title: Fully automated closed-loop two-photon guided patch-clamp electrophysiology *In vivo*

Authors: ***H.-J. SUK**[1], I. VAN WELIE[2], S. B. KODANDARAMAIAH[6,3,4], B. D. ALLEN[3], C. R. FOREST[7], E. S. BOYDEN[3,5,4];
[1]Hlth. Sci. and Technol., [3]Media Lab., [4]McGovern Inst. for Brain Research, Dept. of Brain and Cognitive Sci., [5]Biol. Engin., [2]MIT, Cambridge, MA; [6]Dept. of Mechanical Engin., Univ. of

Minnesota, Twin Cities, Minneapolis, MN; [7]Bioengineering, Georgia Inst. of Technol., Atlanta, GA

Abstract: Two-photon guided patch-clamp is a technique that combines two-photon laser scanning microscopy with whole cell patch-clamp recording. By enabling specific identification of cells and analysis of their subthreshold and synaptic neural activity *in vivo*, it has become a powerful tool for studying the electrophysiological properties of individual neurons in the intact brain. However, performing this technique in a high-throughput fashion is a major challenge, because it involves complicated and laborious procedures that require much skill and experience. We have now, building from our prior development of an automated blind patch system (Nature Protocols 11:634-654, Nature Methods 9:585-587), developed a robotic system that automatically completes the tasks involved in two-photon guided patch-clamp recordings, reducing the need for human expertise and labor.
For automated imaging and identification of fluorescently labeled cells and fluorescent dye-filled pipettes during the patch-clamp process, we have created a Matlab-based module that runs in parallel with ScanImage, an open-source software package commonly used for image-guided patch-clamp recordings *in vivo*. Our system fully automates pipette movement onto the target cell, gigaseal formation, and break-in, by dynamically controlling an automated pressure box and a micromanipulator. Importantly, the pipette movement occurs in a fully closed-loop fashion, adjusting the pipette trajectory according to the target cell movements that are automatically captured by the two-photon microscope images. We tested our automated patch-clamp strategy on fluorescently labeled parvalbumin-positive interneurons in the mouse cortex, and we were able to achieve the whole-cell configuration that enabled recordings of the spiking and subthreshold electrical activity of these cells. Our system has the potential to become a useful tool for skilled electrophysiologists as well as for those new to the field, as it eliminates several laborious manual procedures involved with the two-photon guided patch-clamp process. Furthermore, our system may be scalable to the recording of many cells, if the code and algorithms for all of the automated procedures are extended to multi-cell patch-clamp recordings.

Disclosures: H. Suk: None. **I. van Welie:** None. **S.B. Kodandaramaiah:** None. **B.D. Allen:** None. **C.R. Forest:** None. **E.S. Boyden:** None.

Poster

752. Cellular and Network Electrophysiological Approaches

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

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Topic: I.04. Physiological Methods

Support: NIAAA R01AA016852

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T32NS73553-4

Wake Forest Department of Neurology

Center for Biomolecular Imaging

WFSM Translational Sciences Institute

P40OD010965

Title: Reconstruction of optogenetically induced oscillations using magnetoencephalography

Authors: *G. ALBERTO[1,2], D. KLORIG[2], J. STAPLETON-KOTLOSKI[3], G. POPLI[3], C. CONSTANTINIDIS[2], J. DAUNAIS[4], D. GODWIN[2];
[2]Neurobio. and Anat., [3]Neurol., [4]Physiol. and Pharmacol., [1]Wake Forest Sch. of Med., Winston Salem, NC

Abstract: Oscillatory activity has been observed across nearly every system in the mammalian brain at a wide range of spatial and temporal scales. Oscillations are suspected to have a functional role in the organization, transmission, and processing of information. However, measuring oscillations often requires a tradeoff between spatial and temporal resolution. The temporal resolution afforded with electrophysiological recordings is offset by its restricted anatomical coverage; whereas the whole brain coverage granted with functional MRI does not provide sufficient temporal resolution to capture the transient and high-frequency nature of most oscillations. Magnetoencephalography (MEG) is a whole brain neurophysiological recording technique that allows for both high temporal and spatial resolution when appropriate analytics are applied. We have developed a method that allows for optically generating oscillatory activity in a non-human primate while recording MEG data. We demonstrate that we can localize the site of evoked signals and reconstruct accurately and precisely spectral features of the recorded neural activity with the beamformer SAM, which allows time series-based analysis of oscillations at discrete anatomical sites across the whole brain. CA3 of hippocampus and prefrontal area 8 of vervet monkeys were transfected with AAV10-CaMKIIα-ChR2-eYFP and an optrode was implanted at the transfection site, allowing for simultaneous optical stimulation and electrophysiological recordings. Animals were optically stimulated with 3 seconds of theta (8Hz) or gamma (40Hz) frequency modulated light source. This stimulation pattern elicited a neural response at the frequency of stimulation (8 and 40 Hz) as recorded by the implanted electrode. The same stimulation pattern was then applied during MEG recording. MEG data were analyzed using the beamformer Synthetic Aperture Magnetometry (SAM) from MISL. First, the beamformer was used to identify coordinates of peak activity concordant with the known site of activation. After identifying peak generators a weight matrix was calculated for that location with the same temporal resolution as the MEG recording. This weight matrix was used to calculate an estimated source series, or virtual electrode. Comparison of the directly recorded

electrode data with the source series showed that the source series accurately estimates optically evoked oscillatory behavior. The ability to generate and reconstruct neural oscillations across the whole brain at temporal scales relevant to neural function will allow for testing hypotheses related to the role of native oscillations.

Disclosures: **G. Alberto:** None. **D. Klorig:** None. **J. Stapleton-Kotloski:** None. **G. Popli:** None. **C. Constantinidis:** None. **J. Daunais:** None. **D. Godwin:** None.

Poster

752. Cellular and Network Electrophysiological Approaches

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Topic: I.04. Physiological Methods

Support: NSFC grant 61473169

Title: Functional network parcellation of human cortex using ECoG

Authors: Y. YAN[1], T. QIAN[1,2], W. ZHOU[3], Z. LING[4], H. LIU[2], *B. HONG[1];
[1]Tsinghua Univ., Beijing City, China; [2]Dept. of Radiology, Harvard Med. Sch., Charlestown, MA; [3]Dept. of Neurosurg., Yuquan Hospital, Tsinghua Univ., Beijing, China; [4]Dept. of Neurosurgery,, PLA Gen. Hosp., Beijing, China

Abstract: Large-scale cortical networking patterns have been established based on the correlation of slow fluctuations of resting BOLD signals. However, its electrophysiological basis remained to be elucidated. For the slow nature of hemodynamic signals, the evolution of these networks during rest and tasks is hard to be captured. Here, we developed a novel approach for functional networks parcellation on the basis of the probability of spontaneous co-activation among ECoG electrodes. The accuracy of this parcellation approach was verified by electrical cortical stimulation (ECS) and somatosensory evoked potentials. A further analysis revealed that the brain-wide connectivity is likely built on the ECoG synchrony of power envelop at a common carrier frequency ranging from alpha to low-beta (8-22.5Hz) with slight individual difference. And the synchronization frequency approximated the slow fluctuation (<0.1 Hz) of resting BOLD signals. The high similarity between the above functional network parcellation and the fMRI resting network atlas in individuals also suggested the power-envelope synchrony of neural oscillations as the electrophysiological basis of spontaneous BOLD signals. Furthermore, the slow carrier frequency (8-22.5Hz) based functional network parcellation atlas was found to be consistent across various tasks, and to resemble the resting networks. However, the networking patterns in tasks could be easily distinguished from each other using the carrier

frequency of high gamma band (60-140Hz) instead. This may suggest a common networking mechanism of task and resting brain, with only difference in carrier frequency.

Disclosures: **Y. Yan:** None. **T. Qian:** None. **W. Zhou:** None. **Z. Ling:** None. **H. Liu:** None. **B. Hong:** None.

Poster

752. Cellular and Network Electrophysiological Approaches

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Topic: I.04. Physiological Methods

Support: Marie-Curie FP7 NAMASEN ITN - 264872

Italian Institute of Technology (IIT)

Title: High information content on-chip read-outs based on large-scale monitoring of neural network activity in healthy and diseased conditions

Authors: ***H. AMIN**, F. MARINARO, T. NIEUS, A. MACCIONE, D. DE PIETRI TONELLI, L. BERDONDINI;
Fondazione Inst. Italiano Di Tecnologia (IIT), Genova, Italy

Abstract: Over the past decades, notwithstanding remarkable advances in biotechnology and neurotechnologies, progress in elucidating brain disorders and in the development of novel therapeutic strategies has stalled. Among the different research directions that need to be progressed in parallel to face this challenge, a valuable approach consists in the development of efficient predictive *in vitro* methodologies that exploit emerging neurotechnologies to support elucidating the functional responses of neural circuits involved in different pathophysiological processes. Specifically, we target cell-based assays enabling to better understand the neurophysiology of networks and to pinpoint neurodevelopment and neurodegenerative disease mechanisms, as needed to establish simple, yet well-controlled, disease models for cell therapies and functional drug screening.
In this respect, the confluence of new emerging technological approach realized with low-power CMOS active circuits has been recently introduced. This enables recordings of spiking activity simultaneously from several thousands of densely integrated electrodes (i.e. 4096 electrodes). The result is a unique sensing capability that provides access to extracellular signals at multiple scales in large-scale neuronal networks cultured on-chip.
As it will be presented here, CMOS-MEAs can be applied for studying neuronal ensembles

(murine or human-derived) and their response (with single-neuron detail) to chemicals and drugs as well as for assessing developmental impairments of neuronal spiking activity in genetic models of diseases. On the other hand, high-content imaging (HCI) is combined with these electrical measures to provide multiple cellular measurements from each single experiment. These multimodal read-outs will be demonstrated: i) to assess the use of neural stem cells and of potential biochemical therapies to rescue the early activity-dependent degeneration induced by the toxicity of Aβ oligomers on primary rat hippocampal neurons; ii) to characterize neurodevelopmental alterations of the *in vitro* development of hippocampal neuronal networks from a disease mouse model, in particular, focusing on homeostatic plasticity properties, and iii) to study the development of electrical activity and evoked responses of human-derived neuronal networks over several months.

Disclosures: H. Amin: None. **F. Marinaro:** None. **T. Nieus:** None. **A. Maccione:** None. **D. De Pietri Tonelli:** None. **L. Berdondini:** None.

Poster

752. Cellular and Network Electrophysiological Approaches

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Program#/Poster#: 752.17/MMM37

Topic: I.04. Physiological Methods

Title: Modular and patterned 3D cortical networks on microelectrode arrays as a brain on a chip model

Authors: *R. HABIBEY, A. BLAU;
Italian Inst. of Technol. (IIT), Genova, Italy

Abstract: The brain is composed of modular sub-networks with defined connectivity patterns, a key feature to let higher cognitive capacities emerge. Substrate patterning and physical confinement allow for structuring of neural networks *in vitro* to mimic a brain-like modularity. To this end, we paired a polydimethylsiloxane (PDMS) multi-well microchannel device with microelectrode arrays (MEAs) to connect 64 3D cortical network nodes through four microchannels each (Fig. 1C). Thin (100 μm) PDMS devices were fabricated by soft lithography (1). Each device included 8x8 microwells (h=100 μm, r=80-120 μm) and interconnecting microchannels (h=5 μm, w=20 μm or 30 μm, l=80-120 μm). Microwell were paired with recording electrode (Fig. 1 A and B). Rat cortical neurons were seeded at different densities (~ 10, 50 and 100 cells/well). 3D structures did not develop in low-density modules. However, in dense cultures 3D neural structures appeared in each module residing on a glial carpets at their bottom (Fig. 1G and H). Extracellular signals in patterned cultures appeared around 8 DIV, first

as single spikes, which then turned into burst activity around 14 DIV. The signal amplitudes in patterned cultures were significantly higher than in random cultures (Fig. 1I). The connectivity density between modules varied with the microchannel width and the overall cell density. The transparent microchannel devices on MEAs not only gave clear optical access to the 3D sub-populations, but created a stable cellular microenvironment for long-term recording and microscopy. Simplifying the 2D neural networks by decreasing the total number of cells by a factor of 10 and by compartmentalizing them into 3D modules coupled to electrodes allowed us to model some aspects of the brain's modularity on a chip and also increased the spatial accuracy of the recorded data. The system can be easily adapted to different experimental paradigms that may include pharmacological and molecular studies on separate modules without affecting other compartments. 1. Habibey, R., et al., Lab on a Chip, 2015. **15**(24): p. 4578-4590.

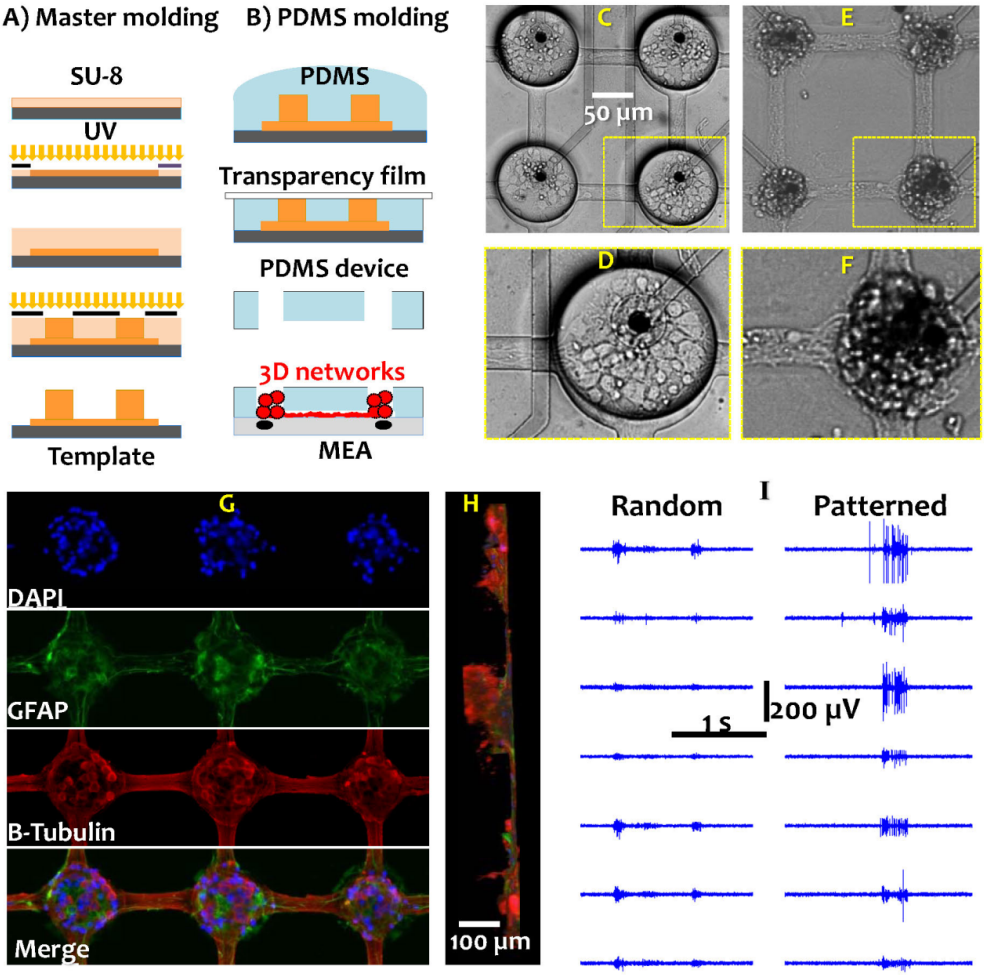

Fig. 1 A) SU-8 template fabrication in five steps and **B)** PDMS microdevice molding in four steps to be aligned with MEA electrodes. **C)** Four network modules at 3DIV; and **D)** magnified view of the outlined module in C. **E)** Four network modules after removing the PDMS device; **F)** magnified view of the outlined module in E. **G)** Immunostained nuclei (DAPI), glial cells (GFAP), neurons (β-tubulin) and merged images. **H)** Lateral view of three reservoir modules with their 3D networks. **I)** Exemplary 2 s-recordings from 7 electrodes on random and patterned cortical networks at 45 DIV.

Disclosures: **R. Habibey:** None. **A. Blau:** None.

Poster

752. Cellular and Network Electrophysiological Approaches

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

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Topic: I.04. Physiological Methods

Support: NIH Grant NS030549

Title: Objective detection criteria and analyses of theta (θ) and gamma (γ) oscillations and their phase-amplitude coupling (PAC) during long-term continuous intrahippocampal LFP recordings in mice

Authors: L. MOLNAR[1], J. DOMOKOS[1], I. FERANDO[2], *I. MODY[2];
[1]Electrical Engin., Sapientia Univ., Targu-Mures, Romania; [2]Dept Neurol., UCLA Sch. Med., Los Angeles, CA

Abstract: In rodents θ oscillations (5-10 Hz) provide a framework for spatial navigation and memory-related tasks, and in single neurons ensure somatic synchronization of dendritic events. During memory tasks the phase (P) of the θ oscillations is coupled to the amplitude (A) of γ oscillations (30-120 Hz), termed PAC. The θ oscillations can be readily detected in local field potential (LFP) recordings, but there is no universal consensus about an objective threshold for their detection. We bilaterally recorded CA1 or dentate gyrus θ and γ oscillations (2048 Hz sampling) in adult mice freely moving in their home cages. Mice with *ad libitum* access to water and food, were kept on a 12:12 hours dark(D):light(L) cycle. Each 24 hrs, recordings were down-sampled (DS) 32× and 8× for detection of θ and γ oscillations, respectively. Using Igor Pro, the DS traces were band-pass filtered with an FIR filter between 5-12 Hz for θ, and 30-120 Hz for γ oscillations. The RMS values of 8 s epochs (0.5 s overlaps) were obtained from the filtered recordings and all-point histograms of θRMS and γRMS values were plotted for 24 hr periods (separately for 12 hr L and 12 hr D). For both θ and γ, the histograms showed a bimodal distribution well fitted by two Gaussians. After testing various thresholds, the point of intersection between the two distributions proved to be most reliable for separating the RMS values belonging to the two Gaussians. Thus, θ and γ oscillatory epochs were identified when RMS values in the epochs were larger than this objective threshold. The presence or absence of θ or γ oscillations were registered in a binary file with "0" for no oscillations and "1" when oscillations were present. Thus, the recordings separately categorized during D and L periods into 4 different types: θ/γ, θ/Nγ, Nθ/γ, and Nθ/Nγ ("N" means "no"). We analyzed the FFT spectra of the 4 types of θ/γ combinations in 8 s epochs of the original recordings sampled at

2048 Hz. Interestingly, peaks on FFTs in the γ range were found in both θ/γ and Nθ/γ types, but not in Nγ. In contrast, peaks in the θ range were only found in recordings where γ was present (θ/γ, and Nθ/γ). When a large θ power was not well delineated from power in neighboring frequencies (δ, and α/β) there was little γ activity. Therefore, a θ oscillation frequency that is clean from contaminations by neighboring frequencies, rather than its absolute amplitude, determines whether simultaneous γ oscillations will be present. Data will be presented on the PAC of P(θ):A(γ) for various θ/γ conditions during L and D cycles as well as the time-dependent alterations and duration of θ/γ epochs over days and weeks.

Disclosures: **L. Molnar:** None. **J. Domokos:** None. **I. Ferando:** None. **I. Mody:** None.

Poster

752. Cellular and Network Electrophysiological Approaches

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Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 752.19/MMM39

Topic: I.04. Physiological Methods

Support: NSF ECCS-1232298

Title: Automatic, reliable and practical approach to extracellular neural action potential sorting using model-based clustering, discriminant model selection, and component agglomeration

Authors: *W. MA, J. SI;
Arizona State Univ., Tempe, AZ

Abstract: Automatic spike clustering without human intervention is critically needed as single unit high channel count extracellular recording becomes increasingly available. To effectively process an overwhelming amount of data, a successful automatic spike sorting algorithm is expected to be 1) accurate in inferring spike-neuron lineage; 2) robust to data condition changes; 3) computationally affordable; and 4) objective in outcomes. We propose a novel spike clustering method which aims at meeting the above expectations. The minimum message length (MML) is known to capture costs of fully-specified models in terms of model complexity and goodness of fit. It is therefore theoretically and practically considered to provide statistically consistent results and stably support models of different complexity. These properties are realized in our method according to the following principles: 1) an unnecessary cluster is unlabeled if it leads to minor change in total message length; and 2) the quality of fit term results in a sudden increase in total message length if a true cluster is unlabeled. Initialized to a greater-than-the-true-number of clusters, our algorithm drives the model complexity through an agglomerative process. It infers the optimal number of clusters based on the first derivative of the

total message length over cluster numbers. Parameter estimation is performed by a modified expectation-maximization (EM) algorithm based on MML for multivariate t-mixtures. Performance evaluation of the algorithm was conducted using simulated data from a known t-distribution. Our method was 1) insensitive to inherent free parameters over a wide range of values; 2) able to infer the correct number of clusters despite data condition changes and model uncertainties. We also compared our algorithm with other popular automatic sorting methods: the t-distribution EM in Plexon's Offline sorter, the Superparamagnetic Clustering in Wave Clus (Quiroga et al., 2004) and the robust variational Bayes (Takashi et al., 2012). Our method was performed truly automatic without human intervention while others were fine tuned for improved performance. Comparisons were made using artificial dataset from Wave Clus package, labeled real dataset from Buzsaki lab and our lab. Our algorithm outperformed all other methods because of the highest classification accuracy and lowest variances over 20 runs, even when subject to worsened SNR and increased similarity among clusters. For unlabeled real dataset from our lab, we evaluated sorting quality by spike statistics such as shape, firing rate and inter-spike-interval. Our algorithm was comparable to or more reasonable than other methods.

Disclosures: **W. Ma:** None. **J. Si:** None.

Poster

752. Cellular and Network Electrophysiological Approaches

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Program#/Poster#: 752.20/MMM40

Topic: I.04. Physiological Methods

Support: NSF ECCS-1232298

Title: Automatic procedures for segmenting and tracking hundreds to thousands of individual neurons recorded by wide-field calcium imaging

Authors: S. SHEN[1], R. WU[1], H.-A. TSENG[2], X. HAN[2], *J. SI[1];
[1]Sch. of Electrical, Computer and Energy Engin., Arizona State Univ., Tempe, AZ; [2]Dept. of Biomed. Engin., Boston Univ., Boston, MA

Abstract: Our recent results demonstrated that using wide-field imaging, we were able to capture the concurrent dynamic activity from hundreds to thousands of neurons over millimeters of brain tissue in behaving mice for an extended period of time. Our data showed that a large fraction of anatomically distinct hippocampal neurons responded to discrete environmental stimuli associated with classical conditioning, and that the observed temporal dynamics of transient calcium signals are sufficient for exploring certain spatiotemporal features of large

neural networks. This advance in neurotechnology has provided new capabilities to the investigation of neural circuit function in systems neuroscience. Along with implementing this imaging technique is the problem associated with processing multi gigabytes of data after each recording session. Analyzing such expansive data sets that contain dynamic information of hundreds to thousands of neurons is an overwhelming task, which is nearly impossible by manual operation.
We therefore developed a new, automatic procedure to segment and track single neurons for their dynamic behavior through each experimental session. First, a template image was formulated by filling up each pixel of the template with the maximum intensity value of that pixel throughout the whole session. Based on the distribution of pixel intensities in the template image, the initial threshold value was chosen within the long-tailed region of the resulting histogram. Next, this threshold value is refined in a feedback loop. The threshold value is applied to the template frame, and all resulting regions of interest are collected and collated. Based on the number of regions of interest that meet the predetermined cell area criteria, the threshold value is adjusted to maximize the cell count: a high threshold value will produce fewer segmented cells while a low threshold value will produce segmented cells that include parts of the background and whose cell areas are too large. We tested our automatic single neuron identification and tracking procedures on multiple datasets, and further validated by manual inspection with a subset of data sets. For the dataset that we validated using manual inspection, the algorithm produced no false positives, and the false negative results were due to either purposely removed overlapping cells or the lack of activity during the time window analyzed.

Disclosures: **S. Shen:** None. **R. Wu:** None. **H. Tseng:** None. **X. Han:** None. **J. Si:** None.

Poster

752. Cellular and Network Electrophysiological Approaches

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Topic: I.04. Physiological Methods

Support: KAKENHI 26282222

KAKENHI 26117520

KAKENHI 16H01888

KAKENHI 16K13116

HFSP RGP0036/2014

KAKENHI 16K13116

Title: Hippocampal EEG state-dependent cortical calcium dynamics

Authors: *Y. SHINOHARA, H. HIRASE;
RIKEN, Wako, Japan

Abstract: Phase-locked synchronization between the hippocampus and cerebral cortex has been reported during working memory task and spatial navigation. However, the spatio-temporal dynamics of the hippocampus and wide cortical areas is largely unknown. Functional imaging methods such as PET and fMRI lack the temporal resolution to analyze synchronized neural activities between the hippocampus and various cortical areas. Owing to the large volume conductance, surface EEG recording cannot precisely identify the location of activity source. Our transgenic mouse line (G7NG817), which expresses the calcium indicator G-CaMP7 in astrocytes and the majority of excitatory neurons in the cortex, enables us to observe the temporal dynamics of cortical calcium in 30Hz-100Hz transcranially. Utilizing this mouse line as an experimental tool, we performed simultaneous recording of hippocampal EEGs and imaging of cortical calcium activities under urethane anesthesia. We found that the cortical calcium dynamics was closely correlated to hippocampal EEG status. Calcium activities observed during theta and non-theta states showed distinct spatial and temporal patterns. Cortical calcium level was higher in wide areas of the cortex during the theta state, and the temporal calcium fluctuation was relatively mild. During non-theta states, basal calcium level was lower, but larger cortical calcium elevations that spread over cortical areas co-occurred with hippocampal ripple oscillations. These cortical calcium transients were observed in various cortical areas such as visual, auditory and somatosensory cortex, and showed a distinct spreading pattern at each ripple event. This observation indicates a rapid and dynamical switching of synchronized activities between the cortex and hippocampus during non-theta states.

Disclosures: Y. Shinohara: None. **H. Hirase:** None.

Poster

752. Cellular and Network Electrophysiological Approaches

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 752.22/MMM42

Topic: I.04. Physiological Methods

Title: Investigation of inter-areal communication through targeted electrophysiological recordings

Authors: *P. JENDRITZA, C. M. LEWIS, P. FRIES;
Ernst Struengmann Inst. (ESI) For Neurosci., Frankfurt, Germany

Abstract: In order to understand neural communication between brain areas it is necessary to record activity from functionally connected populations. Here, we demonstrate how large-scale electrocorticographic (ECoG) recordings can be used to map functional properties of visual cortical areas and to target subsequent intracortical recordings. Local field potentials (LFPs) as well as multiunit activity (MUA) can be recorded from the surface of the cortex, enabling the functional characterization of underlying areas, e.g. the estimation of the local retinotopic map. This map can be used to guide the positioning of electrodes and optic fibers for targeted recordings and optogenetic stimulation, respectively. The application of multiple ECoG arrays to characterize distinct areas in a sensory hierarchy, enables targeted recording from functionally related populations. We show that our approach is applicable at different spatial scales and discuss how it could be extended to target injections of drugs or viral vectors and closed-loop feedback experiments in multiple species, including non-human primates.

Disclosures: **P. Jendritza:** None. **C.M. Lewis:** None. **P. Fries:** None.

Poster

752. Cellular and Network Electrophysiological Approaches

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Program#/Poster#: 752.23/MMM43

Topic: I.04. Physiological Methods

Title: Magnetic resonance as wireless power supply for EEG systems

Authors: *J. BERGELER[1], N. V. DE CAMP[2];
[1]Physiol., Universitätsmedizin Mainz, Mainz, Germany; [2]Physiol., Med. Univ. Mainz, Mainz, Germany

Abstract: Wireless EEG systems are of interest for medical applications as well as scientific research, especially in the field of behavioral physiology. One major challenge for the long-term operation of telemetric EEG systems is the power supply, since battery and accumulator technology are not following Moore's law to date. In order to achieve miniaturized, energy autarkic EEG systems, we developed a wireless power supply with resonant coils. In contrast to inductive coupling, which is restricted to very short distances as well as precise alignment of the coils, resonant inductive coupling (also known as magnetic resonance) is useful as mid field power supply, bridging a space in the range of meters depending on the wavelength. Furthermore, resonant inductive coupling does only power coils which are resonant (defined by

the oscillating circuit consisting of a coil and a capacitor). In contrast to inductive coupling without resonance, the power harvesting efficiency of small receiver coils is superior in the case of magnetic resonance. Therefore, our development is extremely useful for future energy autarkic neural implants with the need of strong miniaturization. Our receiver coil has a dimension of 8x15x1mm. The transmitter board has a dimension of 50x50 cm with one power coil and four resonant coils in which the power coil is supplied with up to 10 Watt. Alternating current sine wave frequency is 13,56 MHz (International Industrial, Scientific and Medical Band). It is possible to support several systems in parallel.

Disclosures: J. Bergeler: None. **N.V. de Camp:** None.

Poster

753. Optical Methods: Imaging and Photostimulation

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 753.01/MMM44

Topic: I.04. Physiological Methods

Support: Allen Institute for Brain Science

Title: Open-source software development kit for adaptive optics and optogenetics with a spatial light modulator

Authors: *R. LIU[1], J. BROCKILL[2], P. LEDOCHOWITSCH[2], P. SAGGAU[2];
[1]Allen Inst. For Brain Sci., Seattle, WA; [2]Allen Inst. for Brain Sci., Seattle, WA

Abstract: Liquid crystal spatial light modulators (SLM) have become essential components in wavefront shaping for optical imaging and manipulation of the nervous system. For example, SLM-utilizing techniques such as adaptive optics have been successfully applied to achieve deep imaging of brain tissue with synaptic resolution, and holographic optical stimulation has been employed to perturb neuronal activities.
However, in the setting of a typical neuroscience lab, technical hurdles to develop those tools remain daunting. We will present results from recent efforts to develop a LabVIEW-based open-source software development kit (SDK) for adaptive optics and optogenetics, with the aim to promote the proliferation of new neurotechniques through the open science activities at Allen Institute for Brain Science. The SDK is fully compatible with both the Meadowlark Optics SLMs and the National Instruments hardware bundles for ScanImage 2015. The SDK will incorporate the SLM calibration routines, arbitrary beam steering and refocusing using SLM, hologram generation, and a method to measure wavefronts based on a multi-dither coherent optical adaptive technique.

Different from other open-source software, we intended to provide functional units for each application suited for adaptation and further expansion by the users rather than a comprehensive solution for a particular application.

Disclosures: **R. Liu:** None. **J. Brockill:** None. **P. Ledochowitsch:** None. **P. Saggau:** None.

Poster

753. Optical Methods: Imaging and Photostimulation

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 753.02/DP10 (Dynamic Poster)

Topic: I.04. Physiological Methods

Support: HHMI

Title: Online analysis and perturbation of brain-wide activity in zebrafish

Authors: N. VLADIMIROV, C. WANG, S. NARAYAN, J. FREEMAN, P. J. KELLER, *M. B. AHRENS;
Janelia Res. Campus / HHMI, Ashburn, VA

Abstract: Understanding how sensory signals drive behavior requires a combination of tools for interrogating brain function, including large-scale neuronal recordings, neural perturbations, and large-scale data analysis. Here we combine whole-brain imaging with online perturbation techniques to a) map the tuning of most neurons in the brain of larval zebrafish to behavioral and sensory variables, and b) perform targeted circuit perturbations to test functional roles of the identified populations.
As a model of sensorimotor transformation, we use the optomotor response (OMR), in which larval zebrafish orient and swim along the direction of visual motion. Using regression analysis, we identify, on a brain-wide scale, populations of neurons whose activity correlates with swimming, visual input, or both. With online analysis during data collection, the whole-brain maps are then used as a guide for targeted cell ablations. Several motor-correlated bilateral groups of neurons in the hindbrain, when perturbed, showed strong and reproducible effects on the behavior. Ablating these cells caused a severe reduction in the dynamic range of swim power and swim duration during the OMR. Our method can also be applied with two-photon optogenetic stimulation instead of ablation.
These methods, combined, establish a technique for perturbing brain activity based on whole-brain functional mapping and large-scale online analysis.

Disclosures: **N. Vladimirov:** None. **C. Wang:** None. **S. Narayan:** None. **J. Freeman:** None. **P.J. Keller:** None. **M.B. Ahrens:** None.

Poster

753. Optical Methods: Imaging and Photostimulation

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 753.03/MMM45

Topic: I.04. Physiological Methods

Title: Simultaneous calcium imaging and optogenetic manipulation in freely behaving mice

Authors: *A. M. STAMATAKIS, S. GULATI, M. TRULSON, S. L. OTTE;
Inscopix, Palo Alto, CA

Abstract: Optogenetics has revolutionized systems neuroscience by providing a tool to causally link distinct neurocircuit activity to behavior. In addition, advances in calcium indicators and imaging techniques have led to important discoveries on how various internal and external stimuli drive the activity of distinct neuronal populations. Here, we introduce a miniaturized lightweight microscope that allows for simultaneous cellular-resolution imaging and optical manipulation within the same field of view in freely behaving mice. This microscope contains a 455 nm LED (EX-LED) for excitation of GCaMP and a 590 nm LED (OG-LED) for selective activation of red-shifted opsins. Both LEDs are housed within the microscope and are emitted through the imaging objective. Paired with an implanted GRIN lens, this microscope allows for long-term, cortical and subcortical simultaneous brain imaging and optogenetic manipulation in freely-behaving mice. We found that the red-shifted inhibitory opsins halorhodopsin (NpHR3.0) and Jaws, are minimally activated by the EX-LED in brain slices. In addition, although we observed significant depolarization of somas expressing the red-shifted excitatory opsin ChrimsonR with the EX-LED in brain slices, we observed minimal postsynaptic currents when stimulating terminals expressing ChrimsonR. Thus, we have demonstrated that it is feasible to pair imaging with somatic and terminal inhibition using NpHR3.0 and Jaws. Furthermore, we have demonstrated that it is feasible to pair imaging with terminal stimulation using ChrimsonR. We also found that GCaMP does not significantly respond to OG-LED stimulation in vivo. Finally, we demonstrate the feasibility of simultaneous optogenetic manipulation and calcium imaging in freely behaving mice.

Disclosures: **A.M. Stamatakis:** A. Employment/Salary (full or part-time): Inscopix. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inscopix. **S. Gulati:** A. Employment/Salary (full or part-time): Inscopix. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual

property rights/patent holder, excluding diversified mutual funds); Inscopix. **M. Trulson:** A. Employment/Salary (full or part-time): Inscopix. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inscopix. **S.L. Otte:** A. Employment/Salary (full or part-time): Inscopix. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inscopix.

Poster

753. Optical Methods: Imaging and Photostimulation

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 753.04/MMM46

Topic: I.04. Physiological Methods

Support: NIH grant GM53395

NIH grant NS69720

DFG Postdoctoral Fellowship

Title: Linear two-color uncaging with zero optical crosstalk

Authors: *S. PASSLICK, M. T. RICHERS, G. C. R. ELLIS-DAVIES;
Dept. of Neurosci., Mount Sinai Sch. of Med., New York, NY

Abstract: Multichromatic uncaging of intra- or extracellular signaling molecules is a powerful means to study neuronal physiology. The greatest challenge of multicolor actuation is to minimize excitation crosstalk, allowing activation of a short wavelength absorbing chromophore without activation of its long wavelength counterpart and vice versa.
The majority of caging chromophores show maximum absorbance at short wavelengths (in the UV range), while exhibiting no to very low absorbance at longer wavelengths. In contrast, designing suitable partner chromophores that absorb at longer wavelengths with minimal absorbance in the short wavelength range has proven to be much more challenging.
The aim of our current work was to develop a pair of caging chromophores suitable for dual-color one photon (1P) uncaging with minimal optical crosstalk. Our lab recently synthesized the caging chromophore “DEAC450” which shows maximum absorbance at 450 nm. By modifying its structure, we were able to significantly reduce its UV light absorbance making it a promising candidate for dual-color 1P uncaging.
We attached this new caging chromophore to the neurotransmitter GABA and co-applied it with the short wavelength caged compound dcPNPP-glutamate to CA1 pyramidal neurons of acute

brain slices. Brief full-field illumination with UV light (375 nm, LED) elicited single or multiple action potentials. If this stimulus was preceded by illumination with longer wavelength light (470 nm), the action potentials were reversibly blocked. Detailed analysis irradiating both compounds with varying power and duration at both wavelengths, demonstrated that the power window for crosstalk is virtually infinite at powers used to evoke physiological signals. As a result of the high uncaging efficiency of these molecules, we were able to apply them at very low concentrations, significantly reducing the antagonism of both compounds against $GABA_A$ receptors. Another advantage of the high uncaging efficiency and the LED full-field illumination used was that UV-uncaging showed little to no cytotoxicity even after many (>100) uncaging pulses.
In summary, we were able to develop a long wavelength caging chromophore that shows so little absorption of short wavelength light that 1P/1P uncaging is possible with zero optical crosstalk.

Disclosures: **S. Passlick:** None. **M.T. Richers:** None. **G.C.R. Ellis-Davies:** None.

Poster

753. Optical Methods: Imaging and Photostimulation

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Topic: I.04. Physiological Methods

Support: NSF IOS 1120938

NIH EY-022122

Title: ProjectorScope 2: an updated optical system for patterned optogenetic stimulation and intrinsic signal imaging

Authors: ***S. WANG**, J. J. OSIK, S. D. VAN HOOSER;
Biol. Dept., Brandeis Univ., Waltham, MA

Abstract: Understanding the operating principles of the cortex is critical to understand the mechanisms by which neural circuits enable perception and behavior, and to understand how these mechanisms go awry in diseases of the nervous system, such as in epilepsy and autism. Optogenetic tools have progressed to enable neuroscientists to directly manipulate neuronal networks with high temporal resolution, opening a new avenue by which to unravel the underlying mechanisms of neural systems by stimulating neurons with light.
My colleagues and I previously developed an optical projection system, called “ProjectorScope 1”, which can play “movies” to the brain surface in intact animals to optogenetically activate

different cortical areas in space and time in order to dissect the role of individual circuit components in neural computation. Recently, we have made several improvements to the optical system and updated a new version, “ProjectorScope 2”, which will further let us perform brain surface imaging to identify functional brain areas, e.g. orientation columns of ferret visual cortex, and subsequently activate these local areas with spatiotemporal control in intact animals that express optogenetic channels.
The new optical system includes a commercial projector that functions as an image generator, and a custom made single-reflex lens connector that replaces the native projection lens and so receives light directly from the projector’s inside prism. This helps to eliminate the misalignment between the projector and the external lenses and thus greatly reduce the optical aberrations. Two juxtaposed achromatic lenses of 30 mm focal length are in place to further minify the image so that the size of the projection area, approximately 4 mm by 4mm, matches the size of the brain surface imaging area. Four identical single-reflex lenses are used in the system to achieve parfocality between the imaging camera and the projector. These adjustments grant ProjectorScope 2 the capabilities of simultaneous projection and imaging. The system can provide 8-11 mW/mm^2 power on the brain surface from a single blue channel that will be sufficient to activate channelrhodopsin-2 channels.
Preliminary results have shown that this system is able to perform brain surface imaging to identify orientation columns of ferret visual cortex and project image masks to subsequently target any patterns of columns. We will use ProjectorScope 2 to assay cortical circuit mechanisms to understand how multiple cortical inputs influence receptive field properties by directly activating local functional areas of the cortex *in vivo*.

Disclosures: **S. Wang:** None. **J.J. Osik:** None. **S.D. Van Hooser:** None.

Poster

753. Optical Methods: Imaging and Photostimulation

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Program#/Poster#: 753.06/MMM48

Topic: I.04. Physiological Methods

Support: AFOSR DURIP Grant

Title: Multimodal nonlinear and thermal imaging platform for assessing infrared neural stimulation

Authors: ***W. R. ADAMS**[1], A. MAHADEVAN-JANSEN[2,3];
[1]Biomed. Engin., [2]Dept. of Biomed. Engin., Vanderbilt Univ., Nashville, TN; [3]Dept. of Neurosurg., Vanderbilt Univ. Med. Ctr., Nashville, TN

Abstract: In recent years, the application of light to modulate neural activity has revolutionized the progression of modern neuroscience research. Infrared neural stimulation (INS), which uses low-energy pulsed infrared light to evoke biochemical and electrical neural responses, is gaining traction as a spatially-selective, artifact-free method of exciting neural activity without genetic modification or introduction of exogenous substances. Current research into the fundamental mechanisms of INS points to a variety of physical and chemical changes that result from a transient local temperature change in irradiated tissue. Despite investigation, the underlying biophysical principles for infrared excitability have yet to be fully elucidated. In efforts to probe these mechanisms, our group has developed a novel microscope that is capable of obtaining label-free, chemically-selective images coregistered with thermal profiles in real time. The microscope combines nonlinear imaging capabilities- including Coherent Anti-Stokes Raman Scattering (CARS), Stimulated Raman Scattering (SRS), Two-Photon Fluorescence (2P), Second Harmonic Generation (SHG)- with real-time temperature distributions through thermal microscopy. CARS and SRS provide a means of real-time, label-free contrast by means of intrinsic, Raman-active vibrational modes present in a particular sample. Two-Photon and SHG imaging allow for label-free probing of cytoarchitecture and metabolic activity alongside standard labelling of physiological dynamics. Coregistration of thermal images with nonlinear imaging provides a unique, real-time perspective of the complex molecular processes during INS. Here we demonstrate the ability of our multimodal microscope to image neural tissue and elucidate molecular changes at the cellular level to provide further insight towards the biophysical mechanisms of INS. Comparison of stimulation paradigms are compared to electrical stimulation. More broadly, we demonstrate the power of applying multimodal, nonlinear, label-free imaging techniques towards neuroscience research to provide unique insight into cellular details and to visualize the dynamics of physiological processes in real time.

Disclosures: W.R. Adams: None. **A. Mahadevan-Jansen:** None.

Poster

753. Optical Methods: Imaging and Photostimulation

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Topic: I.04. Physiological Methods

Support: NIH R01-DC011580

Indiana Clincal and Translational Sciences Institute Pilot Grant

Title: Light gated neurons: The co-varying roles of laser wavelength, power level, and voltage-gated conductances in infrared neural stimulation

Authors: *B. S. COVENTRY[1,2,3], J. T. SICK[4], A. L. SOMMER[2,3], C. A. HADDIX[2], T. M. TALAVAGE[2,5,3], K. M. STANTZ[4,7], E. L. BARTLETT[2,6,3];
[2]Weldon Sch. of Biomed. Engin., [3]Inst. for Integrative Neurosciences, [4]Hlth. and Human Sci., [5]Electrical and Computer Engin., [6]Biol. Sci., [1]Purdue Univ., West Lafayette, IN; [7]Radiology and Imaging Sci., Indiana Univ. Sch. of Med., Indianapolis, IN

Abstract: Electrical stimulation of the nervous system is an invaluable tool for studies in basic science and as an effective therapeutic in cochlear implant and deep brain stimulators. However, use of electrical stimulation is hindered by its propensity for non-specific activation and adverse electro-chemical reactions in neural tissue. Infrared Neural Stimulation (INS) is a relatively new stimulation paradigm which utilizes infrared irradiation in the near infrared range (700-2000nm typically) to evoke excitatory responses in neurons and nerves with higher spatial specificity than electrical stimulation (Izzo et al, 2007) and mitigation of adverse electro-chemical reactions. INS has been applied to diverse areas of the nervous system including sciatic (Wells et al, 2005), auditory nerves (Izzo et al, 2006), and thalamocortical circuits (Cayce et al, 2010) in the central nervous system. Despite these studies, the mechanisms of INS are still not well understood. Furthermore, previous studies were limited in that only single wavelengths at fixed lasing power levels were utilized. In this study, we explore the role of wavelength and power levels in infrared excitation of the rat sciatic nerve and compare these responses to those elicited by electrical stimulation. We found that INS stimulation in the 700-900 nm range, especially at 800 nm, consistently evoked short-latency compound nerve action potentials (CNAP) using very low powers (~10 mW). Additionally, CNAP amplitudes were dependent on laser wavelength and power level, but not energy alone. CNAP amplitude and width were increased by application of the voltage-dependent potassium channel blocker 4-AP and further tests will outline the cellular requirements to evoke the INS-induced CAPs. Concurrently, we explore the ionic basis of INS by selectively blocking sodium, potassium, or TRPV4 channels. Given that INS is wavelength and power level dependent while remaining artifact free, INS laser stimulation parameters can be fine-tuned for a wide variety of basic science and therapeutic applications.

Disclosures: B.S. Coventry: None. **J.T. Sick:** None. **A.L. Sommer:** None. **C.A. Haddix:** None. **T.M. Talavage:** None. **K.M. Stantz:** None. **E.L. Bartlett:** None.

Poster

753. Optical Methods: Imaging and Photostimulation

Location: Halls B-H

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Program#/Poster#: 753.08/MMM50

Topic: I.04. Physiological Methods

Title: Spatially and temporally controlled photomanipulation of neurons

Authors: ***S. JUNEK**[1], J. AULBACH[1], M. FICHTE[2], A. HECKEL[2], A. WINGERT[1], O. WENDT[3];
[1]Max Planck Inst. For Brain Res., Frankfurt Am Main, Germany; [2]Univ. of Frankfurt, Frankfurt am Main, Germany; [3]Rapp OptoElectronic GmbH, Hamburg, Germany

Abstract: Manipulation of cells with light has become a powerful and widely used tool in neuroscience. For this approach to reach its full potential, two conditions need to be met: the precise spatial and temporal control of light delivery inside the sample, and the availability of light-controllable molecules that can modify crucial cellular and neuronal functions.
We present a novel holographic illumination system that can be coupled to existing microscopes (e.g. two-photon microscopes) which provides high resolution control for photomanipulation experiments. The system is optimized for two-photon excitation to improve axial confinement of the excitation patterns as well as tissue penetration. 4d light patterns (x,y,z,t) can be defined by the user based on fluorescence images of the sample.
In addition to a technical characterization of the system we present biological applications of the illumination module using various types of light-sensitive molecules, including caged compounds, photo-switchable ligands of neuronal receptors and optogenetics molecules.

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Poster

753. Optical Methods: Imaging and Photostimulation

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Topic: I.04. Physiological Methods

Support: San PaoloFoundation

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ERC (NEURO-PATTERNS)

Title: Simultaneous fast imaging and manipulation of neuronal networks *In vivo*

Authors: *S. BOVETTI[1], C. MORETTI[1], S. ZUCCA[1], M. DAL MASCHIO[1], P. BONIFAZI[2], T. FELLIN[1];
[1]Italian Inst. of Technol., Genova, Italy; [2]Sch. of Physics and Astronomy, Tel Aviv, Israel

Abstract: Genetically encoded calcium indicators and optogenetic actuators are powerful tools to explore the functional organization of neural circuits. To fully exploit these tools we need a technology that allows fast optical readout of neuronal signals and simultaneous manipulation of network function. However, combing high-speed imaging and optogenetic perturbation simultaneously has been difficult to establish in the mammalian brain because of the poor signal-to-noise ratio that characterizes fast functional imaging and because of potential cross-talk between the different wavelengths that are used for imaging and for optogenetic stimulation. Here, we developed a technique based on patterned two-photon illumination that allows fast (up to 1 kHz) imaging of GCaMP6 signals *in vivo* while manipulating circuit activity with single-photon optogenetic inhibition of Archaerhodopsin (Arch). By combining imaging and electrophysiological recordings *in vivo*, we found that in the scanless configuration single and short trains of action potentials in layer II/III pyramidal neurons could be detected with millisecond precision and improved signal-to-noise ratio compared to the raster scanning approach. Moreover, the artifacts in the fluorescence detection due to single-photon optogenetic illumination were removed in the scanless configuration. As proof of concept, we applied our technique to study the role of parvalbumin-positive (PV) interneurons in the control of spontaneous cortical dynamics. By allowing the readout of the effect of the optogenetic manipulation on neuronal network with unprecedented spatial and temporal resolution *in vivo*, simultaneous high-speed imaging and optogenetics can strongly advance our knowledge of the functional organization of mammalian neural circuits.

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Poster

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Topic: I.04. Physiological Methods

Support: NIH/NINDS Grant R56-NS087249

Title: Tracking compound action potential features to identify areas of inhibition

Authors: *J. FORD[1], M. W. JENKINS[3], H. J. CHIEL[4], E. D. JANSEN[2];
[2]Biomed. Engin., [1]Vanderbilt Univ., Nashville, TN; [3]Pediatrics, [4]Biol., Case Western Reserve Univ., Cleveland, OH

Abstract: Classical analysis of peripheral nerve activity focuses on changes in the compound action potential (CAP). Most studies use the CAP to study motor signals. Some nerves, such as the vagus, contain both motor and sensory axons. We have recently focused on the use of infrared (IR) laser light to block activity in peripheral neuron sub-populations (Jenkins *et al*. at this meeting). It is imperative to analyze CAPs with a method that can quantify changes, including shifts, in the signal's sub-components. Common analysis techniques filter the signal, remove stimulation artifacts, and quantify changes in amplitude, rectified area under the curve, or frequency spectrum. These methods are insensitive to changes in individual CAP features, which are a result of activity changes in axons with differing conduction velocities (indicative of different diameters). In addition, with repeated stimulation, it is often observed that components of the CAP may shift relative to one another. To the best of our knowledge, current tools do not identify CAP features, track those changing features, and identify feature inhibition. Here we present a novel algorithm, implemented in MATLAB, with these capabilities. To test our code, suction electrode recordings from pleural-abdominal nerve in *Aplysia californica* were acquired. CAPs were induced electrically for 37.5 seconds at 4 Hz (150 traces per recording), and features within each CAP were inhibited using a pulsed IR laser (1875nm, 400µm diameter optical fiber, 200Hz pulse frequency, 200µs pulse width, 15 sec duration). Radiant exposures at the fiber output ranged from 123 to 286 mJ/cm^2/pulse to achieve varying degrees of inhibition. First, features (peaks and troughs) were identified in a baseline trace. Features are extracted using the following steps: 1) the signal was smoothed to reduce noise; 2) derivative values were calculated from the slope of a linear fit to a sliding window, reducing the susceptibility to high frequency noise; 3) derivative values were analyzed for sign to identify peaks and troughs; 4) points were only kept if the differences between both neighboring features was above threshold. Extracted features were then tracked through all subsequent traces in the recording using MATLAB's template matching algorithm (Computer Vision System toolbox) to identify features common to all traces. This algorithm is designed to quantify both feature shift and inhibition, comparing baseline and treatment traces to identify inhibited features. Understanding which features are inhibited will help users identify affected populations of neurons and help further guide the application of targeted inhibition.

Disclosures: J. Ford: None. **M.W. Jenkins:** 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; GlaxoSmithKline. **H.J. Chiel:** 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; GlaxoSmithKline. **E.D. Jansen:** None.

Poster

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Topic: I.04. Physiological Methods

Support: NIH R56- NS087272249

AFOSR FA9550-14-1-0303

ASLMS Student Research Grant

Title: A depolarization-activated increase in voltage-dependent potassium currents in response to elevated temperature may account for action potential block in models of the squid giant axon.

Authors: *M. GANGULY[1], M. W. JENKINS[2], H. J. CHIEL[3], E. JANSEN[1];
[1]Vanderbilt Univ., Nashville, TN; [2]Pediatrics, [3]Biol., Case Western Reserve Univ., Cleveland, OH

Abstract: Elevated temperatures are known to inhibit generation and propagation of nerve action potentials in myelinated and unmyelinated nerves. For example, it has been shown that infrared lasers (1.87 μm) that heat the tissue due to water absorption can be used to block action potential propagation in *Aplysia* and in the rat sciatic nerve. A comprehensive computational model will be helpful in deciphering the biophysical mechanisms responsible for infrared block and optimizing parameter space to deliver more efficient/safe block for a variety of different tissues and geometries. We present a computational model that predicts the behavior of an unmyelinated nerve axon, the squid giant axon, that is subjected to elevated temperatures. The computational model was created in NEURON and can be combined with a time-dependent temperature distribution from any heat source. The elevated temperatures used are in the form of spatial and temporal distributions such as those produced during infrared laser heating. Model validation was performed by comparing the effects of change in temperature predicted by the computational model with the published experimental observations in squid giant axon. In response to stimulating currents, regions of elevated temperatures in the squid giant axon show an increase in potassium current that lead to a hyperpolarization of the axon. This depolarization-induced hyperpolarization of the axon may be a significant mechanism for blocking of action potentials at elevated temperatures. Since the standard Hodgkin-Huxley model does not take into account the effects of peri-axonal potassium accumulation or the Na-K pump, we are also exploring their effects on the phenomenon of infrared laser induced thermal inhibition. This model provides us a platform for the preliminary design of a neural interface for selective nerve inhibition that may be used in higher animals, and eventually in humans for chronic pain management.

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Poster

753. Optical Methods: Imaging and Photostimulation

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Topic: I.04. Physiological Methods

Support: GlaxoSmithKline Grant SPN00343

Title: Safety and selectivity of infrared block for small diameter axons.

Authors: ***E. LOTHET**[1], H. LU[1], S. LEWIS[2], M. JENKINS[3], H. CHIEL[4];
[1]Biol., [2]Pediatrics and Pharmacol., [3]Pediatrics and Biomed. Engin., [4]Biology, Biomed. Engin. and Neurosciences, Case Western Reserve Univ., Cleveland, OH

Abstract: Controlling subpopulations of unmyelinated axons within peripheral nerves would make it possible to treat a very wide variety of clinical syndromes. Within the last few years, implanted neural devices have been developed to control peripheral nerve activity. Infrared (IR) light has recently been described as a promising modality for control of neural activity. Previous studies have shown that brief pulses of IR can induce excitation of neurons. Recent studies have shown that IR can inhibit neurons with high spatial specificity. We found that IR light can block nerves with axonal subpopulation selectivity, where smaller diameter axons with slower conduction velocities are more sensitive to the effect of the IR light and are therefore more readily blocked by light than larger diameter axons with faster conduction velocities.
To evaluate infrared settings that can effectively block compound action potentials in an unmyelinated nerve, we used *Aplysia* pleural-abdominal connectives to establish reliability and repeatability of block. We hypothesized that the block will progress through the following events: selective block of smaller-diameter axons, complete block of both small and large-diameter axons with immediate reversibility, and complete block exhibiting irreversibility.
We found that we could rapidly, repeatedly and reversibly inhibit action potential propagation in the slower conducting smaller axons before inhibiting the faster conducting larger axons. At the highest radiant exposure used in these experiments, the shortest delay between the onset of

infrared and partial block of the smaller axons was within 1 second. This delay became longer as we decreased the laser radiant exposure. We also found that it was possible to reversibly block either selectively (only small-diameter, slow-conducting axons) or completely (both slow-conducting and fast-conducting axons) for a prolonged period of time, up to 5 hours.
These results suggest that IR light might be a clinically relevant technique to block unmyelinated C fibers selectively, reversibly and for an extended period of time. In future studies, it may be possible to establish whether this could serve as a novel treatment for significant clinical challenges such as chronic pain.

Disclosures: **E. Lothet:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); GlaxoSmithKline. **H. Lu:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); GlaxoSmithKline. **S. Lewis:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); GlaxoSmithKline. **M. Jenkins:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); GlaxoSmithKline. **H. Chiel:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); GlaxoSmithKline.

Poster

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Topic: I.04. Physiological Methods

Support: NIH Grant 1U18EC021772-01

Title: Infrared light selectively blocks small-diameter fibers

Authors: ***M. W. JENKINS**[1], E. H. LOTHET[2], K. M. SHAW[3], H. LU[3], Y. T. WANG[2], E. D. JANSEN[5], C. C. HORN[6], H. J. CHIEL[4];
[2]Pediatrics, [3]Biol., [4]Biology; Biomed. Engineering; Neurosciences, [1]Case Western Reserve Univ., Cleveland, OH; [5]Biomed. Engin., Vanderbilt Univ., Nashville, TN; [6]Med: Div. of Gastroent, Hepatology, and Nutrition; Anesthesiology; Biobeharvioral Oncology Prog, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Modulation of peripheral sensory nerve signaling is being investigated as a novel treatment for a variety of diseases (e.g., rheumatoid arthritis, pain, obesity, hypertension). Electrical excitation or inhibition of nerve activity is the established approach, but there is a substantial interest in developing techniques to produce greater selectivity in modulating nerve function by targeting specific subpopulations of nerve fibers. Recently, our group demonstrated

that pulsed infrared (IR) light (1860 nm, 200 Hz pulse frequency, 200 μs pulse width) can inhibit peripheral nerve activity with high spatial and temporal specificity. Here, we provide evidence that IR can selectively and reversibly inhibit small-diameter axons at lower radiant exposures than large-diameter axons. We present a mathematical analysis of the cable equation that reveals that modulation techniques primarily affecting the axon surface would preferentially control small-diameter fibers. To validate our mathematical theory, we experimentally demonstrated the effect of infrared light applied to identified neurons of the marine mollusk *Aplysia californica* and in axons within the vagus nerve of the musk shrew. The *Aplysia* model allowed us to directly compare the axonal responses to IR light of two identified neurons (B3 and B43), which have a large and small diameter axon, respectively. IR light consistently blocked B43 at lower radiant exposures (0.0967 J/cm^2 versus 0.131 J/cm^2) than B3 (N=5). We utilized an *in vitro* and *in vivo* Suncus murinus (musk shrew) model to demonstrate selective block in whole vertebrate nerves and small fiber bundles. Again, IR light consistently blocked slow-conducting compound action potentials (CAPs), which correspond to the small-diameter fibers, before blocking faster CAPs (N=3 *in vitro* prep, N=3 *in vivo* prep). Radiant exposures as low as 0.038 J/cm^2/pulse blocked small-diameter fibers. The ability of IR laser to selectively, rapidly, and reversibly control the signaling of small-diameter axons has a large number of applications, including its use as a research tool to investigate the mechanisms of neural circuits and for the treatment of disease.

Disclosures: **M.W. Jenkins:** 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; GlaxoSmithKline. **E.H. Lothet:** None. **K.M. Shaw:** None. **H. Lu:** 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; GlaxoSmithKline. **Y.T. Wang:** None. **E.D. Jansen:** None. **C.C. Horn:** None. **H.J. Chiel:** 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; GlaxoSmithKline.

Poster

753. Optical Methods: Imaging and Photostimulation

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Topic: F.01. Neuroethology

Support: NIH Grant HD075376

Louisiana State Board of Regents RCS 12-180

Title: Gradient index lens implant does not affect behavioral tests and has minimal localized tissue reaction

Authors: *T. A. MURRAY[1], S. A. LEE[1], K. S. HOLLY[1], V. VOZIYANOV[1], I. VLACHOS[2], R. TONG[3], F. G. SZELE[3], S. VILLALBA[4], E. GLASSCOCK[4];
[1]Biomed. Engin., [2]Dept. of Mathematics and Statistics, Louisiana Tech. Univ., Ruston, LA; [3]Dept. of Physiology, Anat. and Genet., Univ. of Oxford, Oxford, United Kingdom; [4]Dept. of Cell. Biol. and Anat., Louisiana State Univ. Hlth. Sci. Ctr., Shreveport, LA

Abstract: In vivo brain imaging in rodent models using multiphoton microscopy (MPM) has greatly enhanced our understanding of the brain. Yet, MPM has depth limitations that generally restricts imaging to a few hundred microns below the pial surface. Implantation of gradient index lenses, or GRIN lenses, has greatly extended the imaging depth to lower cortical layers and even subcortical regions of the mouse brain. Using GRIN lenses, researchers can now visualize dynamic activities, such as Ca2+ signaling, with fine spatiotemporal resolution. Moreover, they can conduct repeated imaging over weeks and months. This presents an opportunity to conduct behavioral tests to correlate performance with changes in network dynamics and cellular structure. Thus, it is critical to know if and how an implanted GRIN lens might affect behavioral test performance. Additionally, it is important to know if the glial scar, which forms around all neural implants, would appear in the tissue being imaged.
To determine the effects of implantation on behavior and imaging, a GRIN lens was implanted in the prefrontal cortex (PFC) of experimental mice, using a minimally invasive surgical technique, and behavioral tests were performed over a seven-week period (three presurgical and five postsurgical sets). Behavioral tests included foot fault, rotarod, and Morris water maze tests. No significant differences were detected between the performance of experimental mice and mice that had either a craniectomy with a cranial window or a sham surgery. Additionally, the 46-micron mean thickness of the glial scar at the bottom of the lens was much thinner than the 125-micron working distance of the lens, which means that scar tissue did not appear in the imaging region. Thus, GRIN lenses can be used for longitudinal imaging in the PFC, and possibly other brain regions, with concurrent behavioral tests in mice as long as the working distance of the GRIN lens exceeds the thickness of the scar and a minimally invasive surgical technique is used.
Reference: SA Lee, et al. (2016) Gradient index microlens implanted in prefrontal cortex of mouse does not affect behavioral test performance over time. PLOS ONE 11(1): e0146533. 22 Jan., 2016.

Disclosures: **T.A. Murray:** None. **S.A. Lee:** None. **K.S. Holly:** None. **V. Voziyanov:** None. **I. Vlachos:** None. **R. Tong:** None. **F.G. Szele:** None. **S. Villalba:** None. **E. Glasscock:** None.

Poster

754. Biomarkers and Drug Delivery Systems

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 754.01/MMM57

Topic: I.05. Biomarker and Drug Discovery

Title: Efficacy profiling of T-817MA, anti-Alzheimer drug candidate, and sigma-1 receptor agonists

Authors: T. YANO, H. TANABE, A. NABETANI, T. OKUDA, *M. NAKAGAWA, T. NAKAMURA;
Toyama Chem. Co Ltd, Toyama 930-8508, Japan

Abstract: T-817MA is an orally available agent, which is in the phase 2 clinical trial for Alzheimer's disease (AD) in the US and Japan. We previously reported that T-817MA recovered cognitive impairment in an AD animal model (Kimura et al., SfN 2006). T-817MA demonstrated a neuroprotective activity against the toxicity induced by amyloid β or oxidative stress such as sodium nitroprusside (SNP). T-817MA promoted neurite outgrowth in reaggregation culture of rat cortical neurons. T-817MA bound to Sigma-1 (σ1) receptor (Ki = 16 nM), which is reportedly involved in oxidative stress resistance and neuritogenesis. σ1 antagonists abrogated efficacy of T-817MA. We hypothesize that T-817MA may exert its pharmacological activity as a σ1 agonist. To compare pharmacological profiles of T-817MA and known σ1 agonists, (+)-pentazocine (Ki = 4.59 nM (Skuza et al., 2003)) and donepezil (Ki = 14.6 nM (Kato et.al., 1999)), neuroprotection assay was undertaken using cultured cortical neurons of rat embryos. T-817MA and (+)-pentazocine showed neuroprotection against SNP-induced cell death (EC_{50} of 5 nM and 500 nM in this study, respectively). Donepezil had no neuroprotection against SNP-induced cell death. In conclusion, T-817MA has potent neuroprotective activity, while other σ1 receptor agonists tested have weak or no activity.

Disclosures: T. Yano: None. **H. Tanabe:** None. **A. Nabetani:** None. **T. Okuda:** None. **M. Nakagawa:** None. **T. Nakamura:** None.

Poster

754. Biomarkers and Drug Delivery Systems

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 754.02/MMM58

Topic: I.05. Biomarker and Drug Discovery

Support: FONDECYT 1131004

FONDECYT 1130531

Title: Mechanism of PAMAM dendrimers internalization in hippocampal neurons

Authors: *F. VIDAL[1], P. VÁSQUEZ[1], C. DÍAZ[2], D. NOVA[1], J. ALDERETE[2], L. GUZMÁN[1]; [1]Dept. of Physiol., [2]Dept. of Organic Chem., Univ. of Concepcion, Concepcion, Chile

Abstract: PAMAM dendrimers are hyperbranched macromolecules which have been described as one of the most promising drug nanocarrier systems. A key process to understand is their cellular internalization mechanism because of its direct influence on their intracellular distribution, association to organelles, entry kinetics, and cargo release. Despite that these mechanisms have been studied in different cell types, in the case of neurons they are not completely described. Considering the relevance of central nervous system (CNS) diseases and neuropharmacology, the aim of this report is to describe the molecular internalization mechanism of different PAMAM dendrimers-based nanocarrier systems in hippocampal neurons. Four dendrimers with different surface properties were studied: G4 with a positive charged surface, PP50 with polyethyleneglycol neutral groups, PAc with acrylate anionic groups and PFO with folic acid molecules. Confocal images show that both G4 and PFO are able to enter the neurons, but not PP50 and PAc. Colocalization study with specific endocytosis markers and specific endocytosis inhibitors assay demonstrate that clathrin-mediated endocytosis would be the main internalization mechanism for G4, whereas clathrin and caveolae-mediated endocytosis would be implicated in PFO internalization. These results show the existence of different internalization mechanisms for PAMAM dendrimers in hippocampal neurons, which opens the possibility to specifically direct them to particular applications and intracellular targets.

Disclosures: F. Vidal: None. **P. Vásquez:** None. **C. Díaz:** None. **D. Nova:** None. **J. Alderete:** None. **L. Guzmán:** None.

Poster

754. Biomarkers and Drug Delivery Systems

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Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 754.03/MMM59

Topic: I.05. Biomarker and Drug Discovery

Support: Support from Solvo Biotechnology

Title: P-glycoprotein (Abcb1a/Mdr1a) limits brain exposure to anti-cancer drug candidate R-roscovitine/seliciclib

Authors: *F. ERDO[1], I. NAGY[2], M. SIKE[3], P. KRAJCSI[2];
[1]Fac. of Information Technol. and Bionics, Pázmány Péter Catholic Univ., Budapest, Hungary; [2]Solvo Biotech., Budaörs, Hungary; [3]CEVA-Phylaxia, Budapest, Hungary

Abstract: Background: Seliciclib (R-Roscovitine) a cyclin -dependent kinase inhibitor, is a promising drug candidate to treat a variety of cancer. R-roscovitine has displayed activity against non-small cell lung-, colon-, breast- and prostate cancer in cell lines. Recently revealed its high activity in patients with chronic lymphatic leukemia or nasopharyngeal carcinoma. Pharmacokinetic studies have shown its high oral bioavailability but limited brain exposure. Rajnai and coworkers reported that seliciclib is a high affinity, selective P-gp substrate *in vitro* in ATPase assay, vesicular transport assay, Hoechst assay, calcein assay and MDCKII-MDR1 monolayer. This interaction is likely to affect its disposition. **Objective**: The aim of our study was to confirm the *in vitro* findings *in vivo* by the investigation of the effect of a specific P-gp inhibitor (PSC-833) on the brain distribution of R-roscovitine in anesthetized and freely moving mice. For making *in vivo-in vitro* (IVIV) correlation a mouse Mdr1a expressing LLC-PK1 monolayer cell line was also applied. **Methods**: To investigate the brain exposure of R-roscovitine dual-probe microdialysis technique was applied in anesthetized and singe-probe microdialysis in freely moving mice. R-roscovitine was administered in a dose of 50 mg/kg i.p. and either vehicle (control group) or PSC-833 (10 mg/kg i.p.) was applied as a pretreatment. Concentration-time profiles were determined in the blood and in the brain and AUC_{brain}/AUC_{blood} was calculated. For IVIV correlation seliciblib alone or LY335979 +seliciclib in combination were tested in LLC-PK1-mock and LLC-PK1-mMdr1a monolayers. **Results:** The brain concentrations of seliciclib increased in a statistically significant manner after co-administration with the P-gp inhibitor in anesthetized mice. The AUC_{brain} values changed from 131 to 327 pmol/mL h ($p<0.05$ by Student t-test compared to the control). In awake animals the AUC value changed from 136 to 242 pmol/mL h in the presence of PSC-833. In the mMdr1a transfected LLC-PK1 monolayer the efflux ratio of seliciclib transport changed from 6 to 1 after incubation with the specific inhibitor LY335979. **Conclusion**: Our results indicate that brain penetration of seliciclib is regulated by the efflux transporter P-gp *in vivo* in accordance with the previous *in*

vitro observations and our IVIV correlation study. The coadministration of seliciclib with a transporter inhibitor may offer a new opportunity for the drug delivery through the blood-brain barrier in case of the treatment of cerebral malignancies.

Disclosures: **F. Erdo:** None. **I. Nagy:** None. **M. Sike:** None. **P. Krajcsi:** None.

Poster

754. Biomarkers and Drug Delivery Systems

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 754.04/MMM60

Topic: I.05. Biomarker and Drug Discovery

Title: Nose-to-brain transport of oxytocin following intranasal delivery with Precision Olfactory Delivery (POD) technology.

Authors: *A. E. BUSTION;
Impel Neuropharma, Seattle, WA

Abstract: Oxytocin (OXT) is a nine amino acid neuromodulatory peptide hormone synthesized in the CNS and in the periphery. Endogenous OXT plays a major role in relationship formation and social function of animals and humans. Hence, exogenous OXT holds potential therapeutic use for disorders that negatively affect social interaction, such as anxiety and schizophrenia. The possible benefits are complicated, however, by OXT's short half-life, metabolic instability, and poor transport across the blood-brain-barrier (BBB). Intranasal (IN) delivery of OXT as a means of bypassing the BBB has been explored both clinically and preclinically, but this is the first time, to our knowledge, that the ability to deliver exogenous IN OXT to different brain regions has been quantified. In the first portion of this study, wildtype C57BL/6 adult mice were dosed with 10 µL (1.2 µg/µL) of radiolabeled OXT (^{125}I-OXT, 2200 Ci/mmol) via the mouse precision olfactory delivery (mPOD) device (Impel NeuroPharma). The pharmacokinetics (PK) and biodistribution of exogenous OXT were assessed at 15, 30, 45, 60 and 90 minutes. In this study, the olfactory bulb exhibited significantly higher concentrations of ^{125}I-OXT compared to other brain regions, indicative of direct nose-to-brain transport along olfactory nerve pathways. Also, the brainstem presented higher peak concentrations than any non-olfactory brain region, suggestive of transport to the CNS along trigeminal nerve pathways. At the cortex and diencephalon T_{max} (30 min), a low pH (pH 4.65) OXT formulation and varying dose levels (0.1 µg/µL, 0.5 µg/µL, and 2.4 µg/µL) were studied to determine the pH sensitivity, linearity, and saturation of absorption of IN OXT. The results showed that OXT absorbs well across the nasal epithelium with no signs of pH sensitivity or saturation of absorption. The dose ranging experiment showed that lower doses result in lower brain levels of OXT, but at a slightly lower

concentration than would be expected if tissue uptake was linearly correlated to the amount of dose administered. This may be due to non-specific binding in the nasal cavity, or to metabolism reducing the rate of uptake at decreased concentrations. In all, this study shows that IN delivered OXT results in nose-to-brain transport and that saturation of uptake is not observed at the dose levels examined.

Disclosures: **A.E. Bustion:** A. Employment/Salary (full or part-time): Impel NeuroPharma.

Poster

754. Biomarkers and Drug Delivery Systems

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 754.05/MMM61

Topic: I.05. Biomarker and Drug Discovery

Support: NIH 1R25MH092912-01

Title: Zirconium phosphate nanoparticle studies on sleep behavior and mortality in *Drosophila melanogaster*

Authors: ***J. J. GORBEA-COLON**[1], N. RODRÍGUEZ[3], B. CASAÑAS[4], J. L. COLÓN[4], J. L. AGOSTO, 00912[2];
[1]Dept. of Biol., Univ. of Puerto Rico - Río Piedras, Guaynabo, PR; [2]Dept. of Biol., Univ. of Puerto Rico - Río Piedras, San Juan, PR; [3]Biol., [4]Chem., Univ. of Puerto Rico Río Piedras, San Juan, PR

Abstract: Drug delivery systems aim to circumvent the difficult process of novel drug discovery by modifying the in vivo activity of known compounds. Zirconium phosphate (ZrP) nanoparticles have shown to be a viable non-cytotoxic platform for the delivery of several drugs including carbamazepine (CBZ). Given the potential medicinal use of ZrP, we test the effects of peroral ZrP administration on Drosophila melanogaster (D. mel) sleep behavior, establish a lethal dose for future experimentation using this combination, and address whether these behavioral effects can be reversed and mortality reduced by discontinuing regimens. We also present studies administering CBZ, a drug known to deprive sleep in D. mel, and CBZ delivered via the ZrP system. To achieve this, ZrP nanoparticles were synthesized from zirconium (IV) chloride and phosphoric acid and assayed using powder X-ray diffraction. For the intercalation of CBZ in the ZrP nanoparticles, stoichiometric amounts of CBZ were added and monitored intercalation by UV-Vis spectroscopy, monitoring total loading efficiency by thermogravimetric methods. ZrP nanoparticles were then suspended in fly food in concentrations of 0.00 mg/mL, 0.25 mg/mL, 2.50 mg/mL and 25.00 mg/mL, while the CBZ-ZrP was loaded at 1mg/mL and CBZ alone was

administered at 1mg/mL as well. The sleep patterns of wild type Oregon R (OreR) flies were assayed under standard Light/Dark (LD) cycles. Assayed flies had continuous access to food stocks for 1 week before exchanging stocks with new ones containing either the same ZrP concentrations or switching their stocks to new food without ZrP, except for CBZ-ZrP and CBZ treatments. After acquiring two weeks' worth of sleep behavior data, analyses were run using MatLab, Prism, and JMP computer software. From these studies, we report that concentrations of up to 2.50 mg/mL were safe in terms of mortality rate. However, in terms of sleep behavior, we observed alterations suggesting that sleep is a more sensitive measure of toxicity. Non-continuous treatment reversed sleep behavior phenotypes in some cases and reduced mortality for some groups. Even though CBZ administration followed previously reported patterns, CBZ-ZrP revealed inconsistent behavior needing further investigation. In conclusion, further studies are needed; however lower ZrP concentrations seem to be a promising alternative for the development of new drug delivery strategies, although biodistribution studies are of utmost importance before moving to next steps.

Disclosures: J.J. Gorbea-Colon: None. **N. Rodríguez:** None. **B. Casañas:** None. **J.L. Colón:** None. **J.L. Agosto:** None.

Poster

754. Biomarkers and Drug Delivery Systems

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 754.06/MMM62

Topic: I.05. Biomarker and Drug Discovery

Title: T-817MA ameliorates cognitive impairment in the Tg2576 transgenic mouse

Authors: *T. OKUDA, T. MIYASHITA, H. SUZUKI, H. KOBAYASHI, T. YANO, A. NAKAMURA, M. NAKAGAWA, T. NAKAMURA;
Toyama Chem. Co., Ltd., Toyama-Shi, Japan

Abstract: T-817MA (1-{3-[2-(1-benzothiophen-5-yl)ethoxy]propyl}azetidin-3-ol maleate), was discovered by Toyama Chemical Co., Ltd. and has been developed as a therapeutic agent for Alzheimer's disease in Phase II clinical study. We previously demonstrated that T-817MA exerted neuroprotective effects against neurotoxicity induced by amyloid β (Aβ) and H_2O_2, and promoted neurite outgrowth in the primary culture of rat neurons (Yamaguchi et al., SfN 2003). Oral T-817MA ameliorated cognitive dysfunctions in the rat intracerebroventricularly infused with Aβ peptide (Kimura et al., SfN 2006). In the present study, we investigated the effect of T-817MA on cognitive dysfunction in the Tg2576 mouse. Tg2576 mice showed progressively impaired cognition in Y-maze test at 14 months of ages, while normal cognition at 5 months. T-

817MA was administered in drinking water at 0.21 and 0.7 mg/mL, corresponding to clinical doses of 112 mg and 224 mg respectively, from 4 to 14 months for the prophylactic treatment. T-817MA in drinking water at 0.7 mg/mL (corresponding to 224 mg) was dosed from 15 to 18 months of ages for therapeutic treatment. In both prophylactic and therapeutic treatments, T-817MA significantly improved memory and cognitive deficits in Y-maze and novel object recognition tests. T-817MA did not change Tris-soluble and insoluble Aβ levels in the brain in all T-817MA dose groups. These results suggest that T-817MA exerts improving effects on cognitive performance without modulating Aβ contents in the Tg2576 mouse.

Disclosures: **T. Okuda:** None. **T. Miyashita:** None. **H. Suzuki:** None. **H. Kobayashi:** None. **T. Yano:** None. **A. Nakamura:** None. **M. Nakagawa:** None. **T. Nakamura:** None.

Poster

754. Biomarkers and Drug Delivery Systems

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Program#/Poster#: 754.07/NNN1

Topic: I.05. Biomarker and Drug Discovery

Support: NIH NS050425

NIH NS058714

NIH NS41509

NIH NS075321

American Parkinson Disease Association (APDA) Center for Advanced PD Research at Washington University

the Greater St. Louis Chapter of the APDA

McDonnell Center for Higher Brain Function

Title: Validation of 6-[^{18}F]fluorodopa PET measures

Authors: ***L. TIAN**[1], S. K. LOFTIN[2], S. A. NORRIS[2], H. P. FLORES[2], J. S. PERLMUTTER[2]; [1]Neurol., Washington Univ. Sch. of Med., Saint Louis, MO; [2]Washington Univ. Sch. of Med., St. Louis, MO

Abstract: Objective: 6-[^{18}F]fluorodopa (FD), an analog of L-DOPA (L-dihydroxyphenylalanine), is a positron emission tomography (PET) tracer used to evaluate

striatal presynaptic dopaminergic function in neurodegenerative diseases, e.g. Parkinson disease (PD). Specific regional brain uptake radioactivity reflects conversion of FD by L-aromatic amino acid decarboxylase (AADC) into ^{18}F-dopamine and retention of this and further metabolites. However, direct comparison of PET-measured *in vivo* regional uptake with local *in vitro* AADC activity has not been done. The purpose of this study is to attempt such validation. **Methods:** Fourteen macaques had baseline MRI and PET using FD, [^{11}C]dihydrotetrabenazine (DTBZ), and 2beta-[^{11}C]carbomethoxy-3beta-(4-fluorophenyl)tropane (CFT), then received unilateral intracarotid MPTP. After several weeks, PETs were repeated. The animals were euthanized at different time points for measurements of striatal AADC enzymatic activities, dopamine concentration. The influx constant (K_{occ}) for FD and non-displaceable binding potential (BP_{ND}) for CFT and DTBZ were calculated for caudate and putamen using an occipital reference region. The AADC enzymatic activity was measured using an enzymatic assay with L-DOPA as substrate using HPLC. **Results:** For all 14 monkeys, the FD K_{occ} strongly correlates with the AADC enzymatic activity in the left caudate (Spearman's correlation $r_s = 0.80$, $p = 0.001$, $n = 14$), ratio of right to left caudate ($r_s = 0.75$, $p = 0.002$, $n = 14$), and ratio of right to left putamen ($r_s = 0.66$, $p = 0.01$, $n = 14$). FD K_{occ} correlates with CFT BP_{ND} in the left caudate ($r_s = 0.59$, $p = 0.06$, $n = 11$), right putamen ($r_s = 0.67$, $p = 0.03$, $n = 10$), ratio of right to left putamen ($r_s = 0.70$, $p = 0.01$, $n = 13$). AADC enzymatic activity correlates with CFT BP_{ND} in left caudate ($r_s = 0.65$, $p = 0.02$, $n = 12$), ratio of right to left caudate ($r_s = 0.73$, $p = 0.005$, $n = 13$), right putamen ($r_s = 0.67$, $p = 0.03$, $n = 10$), ratio of right to left putamen ($r_s = 0.83$, $p = 0.00$, $n = 12$). FD K_{occ} correlates with DTBZ BP_{ND} in the ratio of right to left caudate ($r_s = 0.82$, $p = 0.0001$, $n = 10$), ratio of right to left putamen ($r_s = 0.79$, $p = 0.0002$, $n = 13$). AADC enzymatic activity correlates with DTBZ BP_{ND} in the ratio of right to left caudate ($r_s = 0.69$, $p = 0.01$, $n = 13$), ratio of right to left putamen ($r_s = 0.76$, $p = 0.0001$, $n = 12$). **Conclusions:** Taken together these results show that *in vivo* measured striatal FD K_{occ} reflects in vitro measured striatal AADC activity. Since striatal uptake of CFT and DTBZ correlate with striatal FD uptake, all of these PET measures reflect *in vitro* AADC activity. These findings play a critical role in interpretation of clinical studies using FD PET.

Disclosures: L. Tian: None. **S.K. Loftin:** None. **S.A. Norris:** None. **H.P. Flores:** None. **J.S. Perlmutter:** None.

Poster

754. Biomarkers and Drug Delivery Systems

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 754.08/NNN2

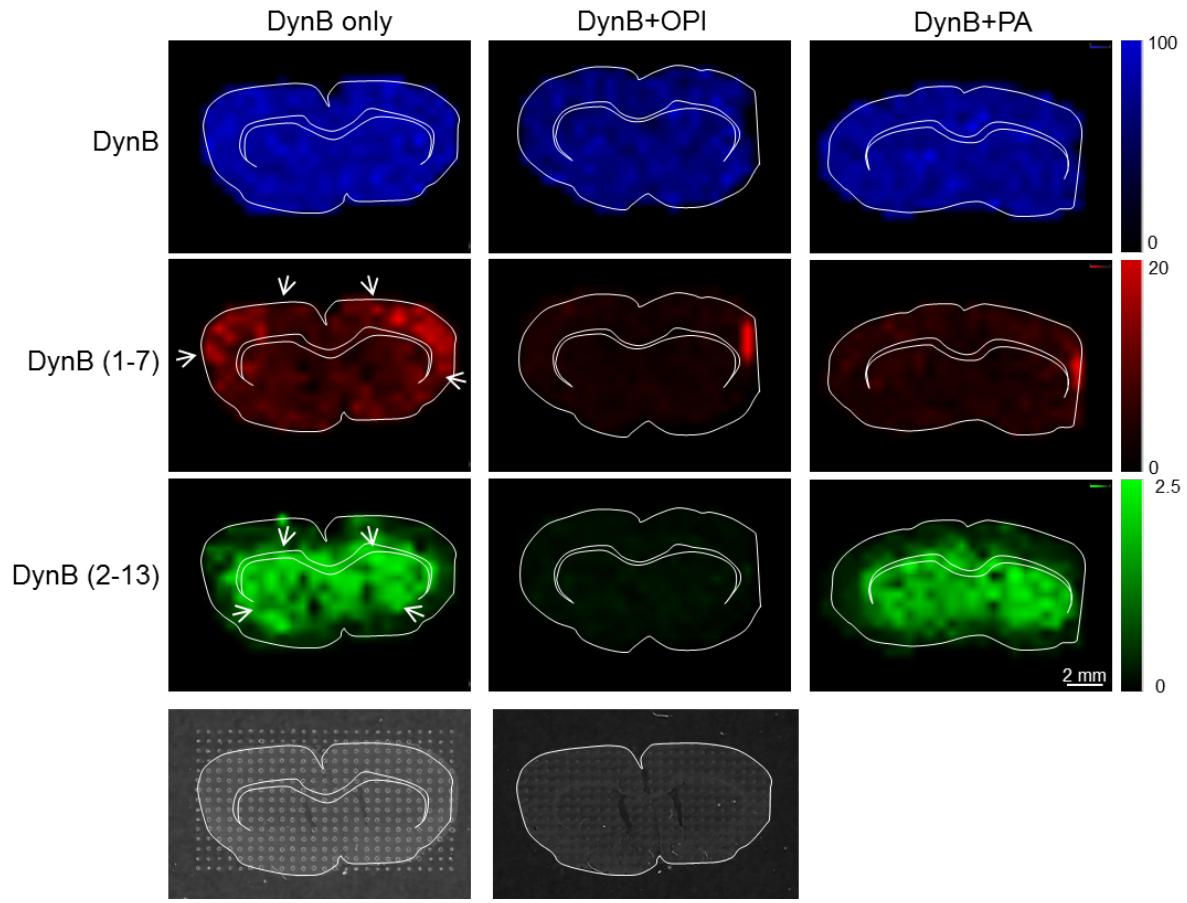
Topic: I.05. Biomarker and Drug Discovery

Support: SRC 521-2012-2304

Title: A new method for detection of region- and enzyme-specific bioconversion of neuropeptides by combining *In situ* histochemistry and MALDI imaging mass spectrometry.

Authors: R. STRÖMVALL, E. BIVEHED, G. TOMASDOTTIR, *M. E. ANDERSSON; Neurotoxicology, Drug Safety and Tox., Dept. Pharm. Biosci, Uppsala Univ., Uppsala, Sweden

Abstract: Region-specific expression of proteolytic enzymes controls the biological activity of endogenous neuropeptides and has recently been targeted for the development of novel drugs, for example in neuropathic pain, depression, and for L-DOPA-induced dyskinesia in Parkinson's disease. Rapid and sensitive analytical methods to profile modulators of enzymatic activity are important for finding effective inhibitors with high therapeutic value.
Here we combined *in situ* histochemistry with MALDI imaging mass spectrometry and demonstrate that this is a highly sensitive method for analysis of brain-area specific neuropeptide conversion of synthetic neuropeptides, and for selection of peptidase inhibitors that differentially target conversion enzymes at specific anatomical sites.
Dynorphin B (DynB) was used as model neuropeptide and effects of peptidase inhibitors applied to brain tissue sections were analyzed. Synthetic DynB (2pmol) was found to be converted to both N-terminal and C-terminal fragments. Several specific and non-specific inhibitors were tested, some of which completely blocked conversion of fragments at 20 attomols on target. We also show dose-dependent inhibition of bioconversion and strain-specific differences in the bioconversion of DynB.
Bioconversion of synthetic DynB was region-specific, producing DynB(1-7) in the cortex and DynB(2-13) in the striatum. Both phosphoramidon (inhibitor of neprilysin) and opiorphin (inhibitor of neprilysin and aminopeptidase N) blocked cortical bioconversion to DynB(1-7), whereas only opiorphin blocked striatal bioconversion to DynB(2-13).
This new unbiased method ISH-MALDI imaging MS will be a very effective tool in the development of novel pharmaceuticals targeting enzyme activity, as it requires no labeling and detects both substrates and metabolites in one single experiment. It can reveal multiple cleavage sites and indicate if several enzymes are involved in bioconversion. Used in profiling mode it is very fast for screening purposes and can also detect the region-specific effects of enzymatic activity.



Disclosures: **R. Strömvall:** None. **E. Bivehed:** None. **G. Tomasdottir:** None. **M.E. Andersson:** None.

Poster

754. Biomarkers and Drug Delivery Systems

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 754.09/NNN3

Topic: I.05. Biomarker and Drug Discovery

Title: Putamen and lateral ventricle adminstration in the cynomolgus monkey

Authors: ***J. DOUVILLE**, G. IACONO, F. EMOND, C. FOUCAULT, J.-F. LAFOND, R. ST-JACQUES, C. COPEMAN;
Charles River Labs. Montreal, Senneville, QC, Canada

Abstract: The putamen along with the caudate nucleus forms the dorsal striatum of the brain. It plays a key role in movement coordination, and in various types of learning. It is involved in many degenerative neurological disorders, like Parkinsons's and Huntington's diseases. Novel therapies, such as large molecules, have low penetration potential due to the blood-brain barrier or due to a short half-life. Therefore, delivery of drugs directly into the target brain compartments may be indicated. In large animals, accurate delivery into a specific brain structure represents a greater challenge. In monkeys, because of marked variability in the size and shape of the head, documented coordinates are considered approximations. To support the development of drugs intended for intracerebral administration, feasibility, reproducibility and tolerability of targeted putamen and lateral ventricle injections were evaluated in 8 terminal and 4 recovery animals. Once the surgical approach was established, a range of volumes were injected bilaterally at different rates to evaluate diffusion and reflux using ink. Following recovery from surgery, the animals were kept for an observation period of 14 days. Clinical signs, body weights, food intake, neurological evaluations, and clinical pathology parameters were monitored to ensure tolerability and evaluate any procedure-related effects. The animals were then euthanized, sequential sectioning of the brain was performed and tissues were embedded in paraffin and stained with H&E for histopathological evaluation. The actual localization of the injection sites, through ink diffusion and needle tracts, was then confirmed by detailed histopathology of the targeted structures. The animals showed no clinical signs related to the injections. There was no effect on body weight or food intake. A standard panel of hematology, coagulation and biochemistry parameters was evaluated and all results were within normal biological ranges. Neurological evaluations showed normal behavior, gait, postural reactions, cranial and spinal nerve functions. The histopathology evaluation demonstrated that the targeted brain structures were reached with minimal background changes. Overall, the putamen and lateral ventricle administration was successfully established.

Disclosures: J. Douville: None. **G. Iacono:** None. **F. Emond:** None. **C. Foucault:** None. **J. Lafond:** None. **R. St-Jacques:** None. **C. Copeman:** None.

Poster

754. Biomarkers and Drug Delivery Systems

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 754.10/NNN4

Topic: I.05. Biomarker and Drug Discovery

Title: Microfluidic manufacture of RNA-lipid nanoparticles leads to highly efficient delivery of potent nucleic acid therapeutics for controlling gene expression

Authors: *G. T. THARMARAJAH[1], E. OUELLET, V6T 1Z3[1], O. SEIRA[2], J. LIU[2], A. THOMAS, V6T 1Z3[1], T. LEAVER, V6T 1Z3[1], A. WILD, V6T 1Z3[1], Y. LI[3], Y. WANG[3], W. TETZLAFF[2], C. L. HANSEN, V6T 1Z3[4], P. CULLIS, V6T 1Z3[5], J. R. TAYLOR, V6T 1Z3[1], E. RAMSAY, V6T 1Z3[1];
[1]Precision Nanosystems Inc., Vancouver, BC, Canada; [2]Intl. Collaboration on Repair Discoveries (ICORD), [3]Brain Res. Ctr., [4]Physics and Astronomy, [5]Biochem. and Mol. Biol., Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Lipid nanoparticles (LNPs) are used to deliver nucleic acids in vitro and in vivo. Here, we describe the robust manufacture and use of clinical-grade lipid-based nanoparticles for nucleic acids delivery at scales suitable for both in vitro screening and in vivo applications. We have conducted studies to evaluate the merits of the technology and further provide insights for delivering short interfering RNA (siRNA) and mRNA. RNA-LNPs were formulated to encapsulate a potent siRNA directed against PTEN. Exceptional cellular uptake (>98%) with minimal toxicity was observed in both primary rat hippocampal and mixed cortical cell cultures. High transfection efficency (>95%) of the encapsulated material resulted in high-level (>85%) PTEN knockdown within the first 4 hours of a low dose (100 ng/ml) treatment; knockdown was sustained for 21 days. Additionally, more than 80% knock-down of a housekeeping gene was observed in primary rat cortical astrocytes, neurons and human iPS-derived neurons using this technology. Similarly, RNA-LNPs encapsulating mRNA were also found to mediate early (< 4 hours) and sustained gene expression (>75% for 7 days) following a single (500 ng/ml) treatment in primary rat mixed cortical cultures. Strategies for locally administering RNA-LNPs into the brain and spinal cord of adult Sprague Dawley rats were also investigated. Localized injections of PTEN-encaspulated siRNA into the motorcortex resulted in significant and sustained (7 days) knockdown. Similarly, local administration at the site of a cervical spinal cord injury significantly reduced target PTEN expression, 10 days later. Collectively, these studies reflect the simplicity and efficacy of this technology in validating new targeted nucleic acid therapies.

Disclosures: **G.T. Tharmarajah:** A. Employment/Salary (full or part-time): Precision NanoSystems, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Precision NanoSystems, Inc. **E. Ouellet:** A. Employment/Salary (full or part-time): Precision NanoSystems Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Precision NanoSystems Inc.. **O. Seira:** None. **J. Liu:** None. **A. Thomas:** A. Employment/Salary (full or part-time): Precision NanoSystems Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Precision NanoSystems Inc. **T. Leaver:** A. Employment/Salary (full or part-time): Precision NanoSystems Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Precision NanoSystems Inc. **A. Wild:** A. Employment/Salary (full or part-time): Precision NanoSystems Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Precision NanoSystems Inc.. **Y. Li:** None. **Y. Wang:** None. **W. Tetzlaff:** None. **C.L. Hansen:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent

holder, excluding diversified mutual funds); Precision NanoSystems Inc. **P. Cullis:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Precision NanoSystems Inc. **J.R. Taylor:** A. Employment/Salary (full or part-time): Precision NanoSystems Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Precision NanoSystems Inc. **E. Ramsay:** A. Employment/Salary (full or part-time): Precision NanoSystems Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Precision NanoSystems Inc..

Poster

754. Biomarkers and Drug Delivery Systems

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 754.11/NNN5

Topic: I.05. Biomarker and Drug Discovery

Support: NINDS

Title: Neuronal autophagy stimulation by nanostructured oxygen solutions.

Authors: *M. V. IVANNIKOV, M. SUGIMORI, R. R. LLINAS;
Neurosci. and Physiol., NYUSOM, New York, NY

Abstract: Neurons rely on basal macroautophagy for protein quality control. Reduction in cellular ATP levels in cells, such as during starvation, potently activates macroautophagy to produce substrates to fuel their mitochondria. Decreased ATP levels in neurons are also a feature of many neurodegenerative conditions, which is mostly the result of mitochondrial inhibition by protein aggregates. Severe declines in neuronal ATP are disruptive to energy-dependent autophagy steps, promoting further protein aggregation and loss of mitochondrial function. Thus, stimulation of mitochondrial or glycolytic ATP output is necessary to boost autophagy to break the cycle and alleviate neuronal protein-aggregate load. In this study, we explore the effects of mitochondrial stimulation by hyperoxic and oxygen nanobubble containing solutions on autophagy in PC-12 cells.
PC-12Adh cells grown in DMEM with 1% horse serum and differentiated with 100 ng/ml nerve growth factor were transfected with pSELECT-GFP-mLC3B construct. Autophagy rate was quantified by monitoring changes in the number of GFP-LC3II puncta using time-lapse microscopy in cells perfused with core HEPES based Tyrode's solution (total observation time ~ 2 h). Perfusion with either hyperoxic Tyrode's solution(HO-T) or Tyrode's solution enriched with oxygen nanobubbles (ON-T) and similar oxygen concentration, ~ 1250 μM, (based on RNS60,

Revalesio, WA) had no effects on autophagy. Mitochondrial oxidative phosphorylation uncouplers 10 μM FCCP or 500 μM MPP^+ with or without ON-T produced no immediate effects on autophagy. Inhibition of mitochondrial respiration with 500 nM antimycin A (AtA), which also increases mitochondrial generation of radicals, also had no immediate effects on the number of autophagosomes (puncta number/cell: control, 36±4; AtA, 37±4; n=10 cells, each). However, perfusion of AtA exposed cells with ON-T but not HO-T led to a dramatic increase in autophagosomes (puncta number/cell: ON-T, 67±13, n=10 cells; HO-T, 31±8, n=8 cells, p=0.021). This together with our previous findings showing mitochondrial ATP synthesis stimulation by oxygen nanobubbles suggests that ON-T upregulates autophagosome formation only during mitochondrial/protein damage. To further confirm that oxygen nanobubbles increase overall autophagic flux, we will image autolysosome formation and acidification by using RFP-GFP-LC3B chimera.

Disclosures: **M.V. Ivannikov:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Revalesio Corp. **M. Sugimori:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Revalesio Corp. **R.R. Llinas:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Revalesio Corp..

Poster

754. Biomarkers and Drug Delivery Systems

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Program#/Poster#: 754.12/NNN6

Topic: I.05. Biomarker and Drug Discovery

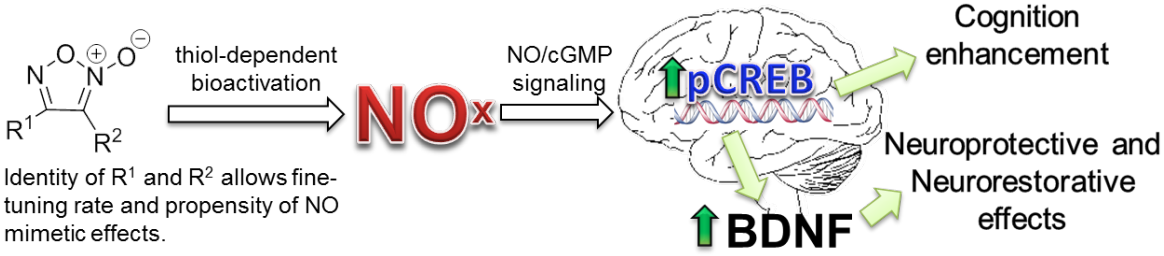
Support: This work was supported by a New Investigator Award from the American Association of Colleges of Pharmacy

This work was supported by a grant from the Alzheimer's Association (NIRG-15-363739)

Title: Discovery of a prototype furoxan as a novel attenuated nitric oxide mimetic for the treatment of neurodegenerative disorders: proof-of-concept studies

Authors: ***I. T. SCHIEFER**, K. NASH, A. KOSTREVSKI, A. NOVAK, A. RAGHAVAN, J. TULSULKAR, Q. ALHADIDI, S. BHATTI, N. WAMER, E. TISHENKEL, B. LANGENDERFER, K. HAGOOD, A. HORTON, E. N. O. TACKIE-YARBOI, Z. SHAH; Medicinal and Biol. Chem., Univ. of Toledo, Toledo, OH

Abstract: Nitric oxide (NO) mimetics capable of enhancing cGMP/CREB signaling have demonstrated efficacy as potential novel therapies for neurodegeneration. Development of NO mimetic chemical classes, often termed "NO donors" or "NO mimetics", has faced challenges centralized around poor pharmacokinetic (PK) profiles which result in risk of adverse effects related to transient spikes in NO signaling, such as acute hypotension. We are developing an NO mimetic class known as furoxans as slow-onset NO mimetics. Furoxans are unique because they exhibit 'tunable' NO mimetic effects. Rate of furoxan NO mimetic effects can be significantly attenuated to produce molecules with superior metabolic stability compared to other NO mimetic chemical classes. We have reported furoxan efficacy in multiple *in vitro* and *ex vivo* systems, including reversal of $A\beta_{42}$ induced synaptic dysfunction in LTP and protection against oxygen glucose deprivation (OGD), a cellular model of ischemia. The present study represents the proof-of-concept examination of furoxans to demonstrate *in vivo* neuromodulatory activity and potential as a novel therapeutic class for the CNS. Our drug development efforts have resulted in the discovery of a prototype attenuated furoxan, designated **IS-1-41**. *In vitro* neuroprotection against OGD and cGMP/CREB target pathway engagement endorsed the advancement of **IS-1-41** to *in vivo* studies. A focused PK study confirmed brain bioavailability and a favorable duration of CNS exposure. In a model of memory, **IS-1-41** provided dose-dependent improvement of scopolamine induced hippocampal-dependent memory deficits in step-through passive avoidance (STPA). In a model of stroke, **IS-1-41** improved motor function and reduced infarct volume when administered following middle carotid artery occlusion (MCAO). Cumulatively, our data supports our hypothesis that an attenuated furoxan can possess the beneficial neuromodulatory effects seen previously for other NO mimetics whilst possessing superior metabolic stability.



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Poster

754. Biomarkers and Drug Delivery Systems

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 754.13/NNN7

Topic: I.05. Biomarker and Drug Discovery

Support: CONACYT-FONSEC SALUD-2012 Grant Number 181381

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Elizabeth Romero has a CONACyT doctoral fellowship and data in this work is part of her doctoral dissertation in the Posgrado en Ciencias Biomedicas de la Universidad Nacional Autónoma de México

Title: No association between levels of uric acid and the rs1014290 polymorphism in Mexican subjects with Parkinson's Disease

Authors: *E. ROMERO GUTIERREZ[1], G. HARO HERRERA[2], E. HERNANDEZ-MENDEZ[2], J. SALAS-PACHECO[2], O. ARIAS-CARRION[3];
[1]Inst. De Fisiología Celular, Univ. Nacional Autónoma De México, Ciudad DE Mexico, Mexico; [2]Univ. Juarez del Estado de Durango, Mexico, Durango, Mexico; [3]Univ. Nacional Autónoma de Mexico, Inst. de Fisiología Celular / Hosp. Gen. Dr. Manuel Gea González, Ciudad de Mexico, Mexico

Abstract: Mexico is dramatically facing the problem of population aging, which has led to an increase in epidemiology of Parkinson's disease (PD). PD is characterized by changes in motor functions due to the death of dopaminergic neurons in the substantia nigra pars compacta. The etiology and pathophysiology of this disease suggest that oxidative stress plays an important role. In this context, antioxidants such as uric acid (UA) could have neuroprotective properties. Low UA levels have been proposed as a risk factor for PD and its progression. In light of these findings, the rs1014290 single nucleotide polymorphism (SNP) in the gene SLC2A9 has been linked to the reduction of UA.
The aim of this study is to determine the association between UA levels and the rs1014290 SNP in a case-control study. The case group included 59 PD patients, while the control group included 169 non-PD subjects matched by gender and age. The clinical diagnosis of PD was according to the criteria established by The Parkinson's UK Brain Bank. Whole blood samples were taken to determine the serum UA levels and to extract genomic DNA. The rs1014290 SNP was genotyped by qPCR.
Our results show that UA serum levels are not significantly different (4.7 ± 1.13 vs 4.8 ± 1.39, $p = 0.652$) in case group vs control group. In the analysis stratified by sex, we did not find significant differences in the levels of UA compared with controls, neither for women nor men

with PD (4.3 ± 1.25 vs 1.15 ± 4.5, p = 0.449 in women and 5.15 ± 1.27 vs 5.21 ± 1.56, p = 0.87 in men). The allele frequency of the mutated variant was increased in patients with PD, but no significant difference was observed vs controls (33.3 vs 29.16 %, p = 0.476). Regarding the genotype frequencies for the mutated homozygous and heterozygous alleles, there was also an elevation in the case group relative to the control group, however the difference was not significant (9 vs 11% and 40 vs 49 %, p = 0.88).
This is the first study exploring the association between levels of UA and the rs1014290 SNP of the SLC2A9 gene in Mexican subjects with PD. Our findings indicate that levels of UA and the rs1014290 polymorphism might not be associated in PD. Nevertheless, the number of patients needs to be increased in order to draw stronger conclusions.

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Poster

754. Biomarkers and Drug Delivery Systems

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 754.14/NNN8

Topic: I.05. Biomarker and Drug Discovery

Support: NIH R43 MH110301

Title: Rapid phenotypic and functional maturation of iPSC-derived human neurons for drug discovery

Authors: *Z.-W. DU[1,2], M. HENDRICKSON[1], B. DUNGAR[1], S.-C. ZHANG[2];
[1]BrainXell Inc., Madison, WI; [2]Waisman Ctr., Univ. of Wisconsin, Madison, WI

Abstract: Neurons derived from human induced pluripotent stem cells (iPSCs) represent a tremendous opportunity to create platforms for drug discovery with a high relevance to human psychiatric and neurological diseases. iPSCs can be generated from both healthy individuals and from patients with a diagnosed CNS disorder. Indeed, for patients harboring a known genetic mutation, isogenic control iPSCs can also be created using gene-editing technologies such as CRISPR. A range of neuronal subtypes can then be generated from these iPSC lines. However, implementation of this approach has been slowed due to long maturation times for cultured human neurons. Here, we demonstrate the rapid maturation of four subtypes of neurons derived from human iPSCs: (1) spinal motor neurons, (2) midbrain dopaminergic neurons, (3) cortical glutamatergic neurons, and (4) cortical GABAergic neurons. These neurons mature within 7-14 days after plating as evidenced by diverse measures. They display a complex morphology,

including extensive neurite branching. The neurons express classical subtype-specific markers as well as pre- and post-synaptic proteins. Finally, they show functional activity as demonstrated by robust spiking in multi-electrode array (MEA) studies and calcium imaging. As a proof-of-concept for high throughput screening, we knocked a nanoluciferase reporter into the survival motor neuron gene in iPSCs derived from spinal muscular atrophy patients. Motor neurons from the reporter SMA iPSCs respond to positive compounds in a dose-dependent manner in a 384-well format. Given that these neurons can be produced in high purity (>70-90%) and mature in approximately one week, they provide a powerful system for CNS drug discovery and development.

Disclosures: **Z. Du:** A. Employment/Salary (full or part-time): BrainXell Inc. **M. Hendrickson:** A. Employment/Salary (full or part-time): BrainXell Inc. **B. Dungar:** A. Employment/Salary (full or part-time): BrainXell Inc. **S. Zhang:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); BrainXell Inc..

Poster

754. Biomarkers and Drug Delivery Systems

Location: Halls B-H

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Program#/Poster#: 754.15/NNN9

Topic: I.05. Biomarker and Drug Discovery

Support: NIH Grant 5R21NS084492

Title: Mems based gene and drug delivery system: voltage controlled accumulation and release of molecules

Authors: S. SAMPATH KUMAR, A. MOORE, A. SRIDHARAN, *J. MUTHUSWAMY; Dept Bioengineering, Arizona State Univ., Tempe, AZ

Abstract: Delivering genes to targeted neurons in the brain is critical for the success of gene therapy and basic neurophysiology studies focused on understanding the functional role of specific brain circuits. However, current non-viral gene delivery technologies are inefficient and imprecise in spatial localization. We report a novel MEMS based system for targeted delivery, which can also facilitate automation of the process. Polycrystalline silicon nanoelectrodes were milled using focused ion beam (FIB) to enable delivery of payloads into single neurons. A voltage controlled method was optimized that allows for immobilization of a payload to the tip of the electrode in a way that a) prevents any loss of payload until the electrode penetrates the neuron; and b) allows for controlled injection and release of the payload inside the neuronal cell

body. Payloads of two different sizes were tested - scrambled siRNA conjugated with Alexa Fluor and fluorescein dye conjugated with dextran. First, glass micropipettes were used to determine the parameters required for reproducible charge-mediated accumulation and release of payloads. Next, the parameters to achieve efficient voltage controlled accumulation of payloads onto the polysilicon nanoelectrodes and their release were optimized. Finally, ability of these electrodes to achieve controlled delivery into targeted neurons of abdominal ganglion of Aplysia was tested. Fluorescence imaging confirmed successful delivery of both types of payloads in n = 15 neurons. Propidium iodide staining of the cells confirmed that they were viable after injections with the nanoelectrode. Although preliminary, these results suggest that this technique offers a promising method of single cell gene and drug delivery.

Disclosures: S. Sampath Kumar: None. **A. Moore:** None. **A. Sridharan:** None. **J. Muthuswamy:** None.

Poster

754. Biomarkers and Drug Delivery Systems

Location: Halls B-H

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Program#/Poster#: 754.16/NNN10

Topic: I.05. Biomarker and Drug Discovery

Title: 2-photon evaluation in-vivo of acute microglial response to induced tissue damage in vicinity of implanted neural probe embedded in gelatin loaded with minocycline

Authors: *J. AGORELIUS[1,2], A. D. HOLMKVIST[2], M. FORNI[2], C. E. LINSMEIER[2], J. SCHOUENBORG[2];
[2]Neuronal Res. Ctr., [1]Lund Univ., Lund, Sweden

Abstract: A major issue in the field of neural interface is the foreign body response and the subsequent glial scaring around implanted probes which is believed to lead to electrical insulation and subsequent failure of the implant.
One of the first cells to react to a foreign body is the Microglia, which quickly (within minutes) after tissue damage prolongs its motile processes to the site of damage. One strategy beyond controlling the flexibility and integration of neural probes into the tissue could be controlling the local milieu in the near vicinity of the electrode with local delivery of drugs around the implant. For example minocycline has been shown to inhibit the microglial activity.
In this study, gelatin embedded neural probes was implanted into the cortex of transgenic mice (CX3CR1-GFP knock-in mice) with has fluorescently labeled microglial cells, and a cranial window was created for in-vivo imaging. A controlled and very local tissue damage was induced in the tissue at a fixed distance from the implanted probe, by continuously scanning with high

intensity laser from the 2-photon microscope. And the subsequent acute tissue response was monitored with 2-photon microscopy by collecting z-stack time series to monitor the dynamics of the microglial cells in vicinity of the damage. Quantification of the microglial response was made by comparing the normalized intensity of the region closest to the damage with a region outside as well as by analyzing conformational changes of the individual cells. Probes embedded with pure gelatin was compared to probes with gelatin loaded with minocycline in order to evaluate the minocycline's effect on the acute microglia response to the damage around the implant.

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Poster

754. Biomarkers and Drug Delivery Systems

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Topic: I.05. Biomarker and Drug Discovery

Support: NSF IIP-1353643

NIH 1R43GM106671

NIH 1R43OD020306

USC Coulter Translation Research Grant

Title: Wireless control for simultaneous drug administration in micropump-implanted mice in an array of vivarium cages

Authors: ***T. Q. HOANG**[1,2], M. VAISHYA[1], C. JONES[1], S. STRONKS[1], L. RODRIGUEZ[1], M. MAHDI[3], G. SHACKLEFORD[3], R. CASTRO[1], C. GUTIERREZ[1], J. FIELD[1], A. EPSTEIN[3], R. MOATS[3], E. F. MENG[2,1];
[1]Fluid Synchrony, LLC, Pasadena, CA; [2]Biomed. Engin., USC, Los Angeles, CA; [3]CHLA, Los Angeles, CA

Abstract: For new drug development, advanced biologics and neurologic research, rodent studies requiring chronic drug administration can be very labor intensive and result in dramatic increases in exposure and handling of the animals. To improve both animal care and researchers working conditions, an automated wireless-operated microinfusion system has been developed for the vivarium environment. The system consists of a miniaturized drug pump that is implanted

subcutaneously in each rodent, an external wirelessly inductive controller, and a programmable dosing software. Advantages include minimal handling, fewer interventions, and tether-free dosing for more normal, stress-free behavior. We demonstrated simultaneous microinfusion pumping in mice using an array of external controllers that instrument a standard vivarium cage rack system. A micropump was implanted subcutaneously in the back of transgenic mice expressing the luciferase gene. Each animal was housed in a single vivarium cage and array of six instrumented cages were configured in a vivarium cage rack system. The dosing regimen, bolus dose of ~30 microliter of luciferin solution, was programmed into the dosing software installed on a laptop. Upon command by the software, all micropumps in the mice were activated as indicated by visual observation of the red light shining through the skin from the activated LED (light-Emitting Diode) in each micropump. 90 minutes after the bolus injection, all animals were imaged using a Xenogen bioluminescence imager. Two animals showed comparable bioluminescence intensity levels to those from control animals manually injected with a syringe with similar bolus dose. Electromagnetic interference was identified as major concern for array configuration in the cage rack. Specific strategies to minimize this interference were investigated including synchronized activation, optimal spacing and signal dither. Benchtop evaluation was done by simulating a cage matrix and running up to 6 pairings of pump-controller simultaneously in different configurations, and the delivered fluid was measured using micro-pipettes. Our research confirmed the scalability of our array for simultaneous wireless control of multiple pumps to enable a very efficient microinfusion system with high throughput, automated dosing.

Disclosures: **T.Q. Hoang:** A. Employment/Salary (full or part-time): Part-time, Fluid Synchrony, LLC, Part-time, Univesity of Southern California. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Stock, Fluid Synchrony, LLC. F. Consulting Fees (e.g., advisory boards); Consultant, Wallace H Coulter Foundation. **M. Vaishya:** A. Employment/Salary (full or part-time): Full-time, Fluid Synchrony, LLC. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); stock, Fluid Synchrony, LLC. **C. Jones:** A. Employment/Salary (full or part-time): Full-time, Fluid Synchrony, LLC. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Stock, Fluid Synchrony, LLC. **S. Stronks:** A. Employment/Salary (full or part-time): Full-time, Fluid Synchrony, LLC. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Stock, Fluid Synchrony, LLC. **L. Rodriguez:** A. Employment/Salary (full or part-time): Full-time, Fluid Synchrony, LLC. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Stock, Fluid Synchrony, LLC. **M. Mahdi:** None. **G. Shackleford:** None. **R. Castro:** A. Employment/Salary (full or part-time): Part-time, Fluid Synchrony, LLC. **C. Gutierrez:** A. Employment/Salary (full or part-time): Part-time, Fluid Synchrony, LLC, Full-time, Google. **J. Field:** A. Employment/Salary (full or part-time): Part-time, Fluid Synchrony, LLC. **A. Epstein:** None. **R. Moats:** None. **E.F. Meng:** A. Employment/Salary (full or part-time): Full-time, USC, Part-time, Fluid Synchrony, LLC. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual

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Poster

754. Biomarkers and Drug Delivery Systems

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 754.18/NNN12

Topic: I.05. Biomarker and Drug Discovery

Title: Investigations into whether dose volume and exogenous mechanical force can enhance antisense oligonucleotide distribution in the central nervous system following lumbar intrathecal administration in cynomolgus monkeys

Authors: *C. MAZUR[1], D. WOLF[2], B. POWERS[1], J. SULLIVAN[3], J. HOPPIN[3], E. SWAYZE[1], A. VERMA[2];
[1]Neurosci. Drug Discovery, Ionis Pharmaceuticals, Inc., Carlsbad, CA; [2]Biogen, Inc., Cambridge,, MA; [3]inviCRO, LLC, Boston, MA

Abstract: The intrathecal (IT) dosing route has been used to bypass the blood-brain barrier and deliver drugs directly to the central nervous system (CNS). Difficulties with delivering drugs to the brain by intrathecal dosing have been noted due to large caudal to rostral drug concentration gradients. The distribution of drugs administered directly to the cerebral spinal fluid (CSF) has been shown to be affected by CSF pressure differences due to respiration and heart rate. We attempted to model the caudal to rostral drug concentration gradients in monkeys by intrathecal administration of an antisense oligonucleotide (ASO). We tested the effects of different volumes of injection and the application of exogenous mechanical forces, to mimic enhanced respiration effects, on the caudal to rostral distribution of the ASO.
Cynomolgus monkeys (4 groups of N=5) received a lumbar puncture injection of a pharmacological dose of an ASO targeted to the ubiquitously expressed long non-coding RNA MALAT1 in either a low (0.8 mL) or high (2.4 mL) volume of injection, and either with or without immediate application of high frequency chest wall oscillation. A dose of ^{99m}Tc-DTPA was spiked into the formulation and the quality of each lumbar injection was monitored by SPECT/CT. Animals were euthanized 7 days after drug administration and CNS tissues were collected for measurement of tissue ASO concentration and MALAT1 RNA expression by qRT-PCR. CNS tissues were also collected and fixed for immunohistochemistry with an anti-ASO antibody and for in situ hybridization with MALAT1 probes.
The high volume bolus resulted in broader distribution, higher ASO concentrations and decreased MALAT1 RNA expression in the structures of the rostral CNS than did the low

volume bolus. The high volume bolus also decreased local drug concentration in the lumbar spinal cord near the injection site and yielded lower inter-patient variability compared to the low volume dose. The broad distribution resulting from the high dose volume of the ASO was demonstrated by immunohistochemical staining. The MALAT1 expression reduction coincided with these areas of ASO distribution as evidenced by the in situ hybridization. Treatment with exogenous mechanical forces slightly improved distribution in the low volume dose groups in this study.
These results will be used in the future to guide the practices used in the clinic for the IT administration of ASOs directed towards targets involved in CNS diseases.

Disclosures: C. Mazur: A. Employment/Salary (full or part-time): Ionis Pharmaceuticals, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ionis Pharmaceuticals, Inc. **D. Wolf:** A. Employment/Salary (full or part-time): Biogen, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Biogen, Inc. **B. Powers:** A. Employment/Salary (full or part-time): Ionis Pharmaceuticals, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ionis Pharmaceuticals, Inc. **J. Sullivan:** A. Employment/Salary (full or part-time): inviCRO, LLC. **J. Hoppin:** A. Employment/Salary (full or part-time): inviCRO, LLC. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); inviCRO, LLC. **E. Swayze:** A. Employment/Salary (full or part-time): Ionis Pharmaceuticals, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ionis Pharmaceuticals, Inc. **A. Verma:** A. Employment/Salary (full or part-time): Biogen, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Biogen, Inc..

Poster

754. Biomarkers and Drug Delivery Systems

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 754.19/NNN13

Topic: I.05. Biomarker and Drug Discovery

Support: This work was funded as part of the UCL:Eisai Drug Discovery and Development Collaboration Agreement

Title: Identification of ER-000444793, a Cyclophilin D-independent inhibitor of mitochondrial permeability transition using a high-throughput screen in cryopreserved mitochondria

Authors: *T. BRISTON[1], S. LEWIS[1], M. KOGLIN[1], K. MISTRY[1], Y. SHEN[2], N. HARTOPP[1], H. FUKUMOTO[3], M. R. DUCHEN[4], G. SZABADKAI[4], J. STADDON[1], M. ROBERTS[1], B. POWNEY[1];
[1]Eisai Ltd., Hatfield, United Kingdom; [2]Eisai Inc., Andover, MA; [3]Eisai Co. Ltd., Tsukuba, Japan; [4]UCL, London, United Kingdom

Abstract: Growing evidence suggests that persistent mitochondrial permeability transition pore (mPTP) opening is a key pathophysiological event in cell death underlying multiple neurodegenerative disease states. Current agents targeting the mPTP are limited by off-target effects and low therapeutic efficacy, therefore identification and development of novel inhibitors is necessary. A method was developed to cryopreserve large batches of functionally active mitochondria from cells and tissue. Assessment of cryopreserved mitochondria revealed preserved respiratory coupling and ATP synthesis, Ca^{2+} uptake and maintenance of transmembrane potential.
A high-throughput screen (HTS), using an assay of Ca^{2+}-induced mitochondrial swelling using cryopreserved mitochondria identified ER-000444793 as a potent inhibitor of mPTP opening. ER-000444793 was further evaluated using assays of Ca^{2+}-induced membrane depolarisation and Ca^{2+} retention capacity. ER-000444793 failed to affect cyclophilin D (CypD) enzymatic activity, nor did it displace CsA from CypD protein, suggesting a mechanism independent of CypD inhibition.
The ability to maintain mitochondrial function after freeze-thaw provides a platform to perform rapid, large scale compound screens. Here, we identified a novel and CypD-independent inhibitor of mitochondrial permeability transition. The screening flow and compound described will be useful for advancing the search for novel agents targeting mitochondrial permeability transition and understanding the molecular nature of the pore.

Disclosures: **T. Briston:** A. Employment/Salary (full or part-time): Eisai. **S. Lewis:** A. Employment/Salary (full or part-time): Eisai. **M. Koglin:** A. Employment/Salary (full or part-time): Eisai. **K. Mistry:** A. Employment/Salary (full or part-time): Eisai. **Y. Shen:** A. Employment/Salary (full or part-time): Eisai. **N. Hartopp:** A. Employment/Salary (full or part-time): Eisai. **H. Fukumoto:** A. Employment/Salary (full or part-time): Eisai. **M.R. Duchen:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; Research funded by Eisai. **G. Szabadkai:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; Research funded by Eisai. **J. Staddon:** A. Employment/Salary (full or part-time): Eisai. **M. Roberts:** A. Employment/Salary (full or part-time): Eisai. **B. Powney:** A. Employment/Salary (full or part-time): Eisai.

Poster

754. Biomarkers and Drug Delivery Systems

Location: Halls B-H

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Program#/Poster#: 754.20/NNN14

Topic: I.05. Biomarker and Drug Discovery

Support: NIH Grant 5R01A1093504-04

Title: Neuron-specific intracellular delivery of therapeutic cargo with atoxic derivatives of botulinum neurotoxins.

Authors: *P. H. BESKE[1], E. J. CINTRON-VAZQUEZ[2], M. R. EISEN[1], C. ANGELES[3], P. A. BAND[2], P. M. MCNUTT[1], K. ICHTCHENKO[3];
[1]US Army Med. Res. Inst. of Chem. Def., Aberdeen Proving Ground, MD; [2]CytoDel LLC, New York, NY; [3]New York Univ. Sch. of Med., New York, NY

Abstract: Inefficient cytosolic delivery limits the therapeutic utility of many biologic drugs for treating neurotoxic and neurodegenerative disease states. Although recent advances in biologic formulation have achieved some success, more robust strategies are needed for effective transport of biologics to the neuronal cytosol. In this study, we explore the modular protein nanomachine - botulinum neurotoxin (BoNT) - as a prototype neuron-specific vehicle for intracellular delivery of biologic cargo. BoNT contains a 100 kDa heavy chain (HC) and a 50 kDa light chain (LC) that remain associated through a disulfide bond. The C-terminal of the HC mediates highly selective and efficient binding to receptors on the presynaptic membrane of neurons. Following synaptic endocytosis, the N-terminal domain of the HC forms a pore that facilitates translocation of the catalytically active LC through the endosomal membrane to the presynaptic cytosol. The combination of efficient neuronal targeting and potent presynaptic activity renders BoNTs the most poisonous substances known. However, recent studies have shown that point mutations to the active site of the LC can dramatically decrease toxicity while maintaining specific neuronal targeting. The Ichtchenko lab has systematically designed and expressed a library of atoxic BoNT derivatives as prototype vehicles to deliver therapeutic cargo to the neuronal cytoplasm. Here, we provide a functional characterization of the localization and activity of select atoxic BoNT derivatives and their cargo *in vitro* and *in vivo*. In synaptically active cultures of neurons, molecular and electrophysiological readouts of host:toxin activity demonstrated that atoxic BoNT derivatives can successfully deliver diverse biologic cargo to the neuronal cytoplasm. In murine models of botulism, atoxic BoNT-mediated intracellular delivery of single chain antibodies against wild type BoNT/A provided protection from lethality. Protection was provided at post-intoxication treatment times beyond the effective window for cell impermeant antitoxin treatment, thus demonstrating an intracellular mode of action. These studies serve as an important proof-of-concept for future development of atoxic BoNTs as

vehicles for neuron-specific delivery of therapeutic cargo. *Disclaimer: The views expressed in this abstract are those of the authors and do not reflect the official policy of the Department of the Army, Department of Defense, or the U.S. Government.*

Disclosures: P.H. Beske: None. **E.J. Cintron-Vazquez:** None. **M.R. Eisen:** None. **C. Angeles:** None. **P.A. Band:** None. **P.M. McNutt:** None. **K. Ichtchenko:** None.

Poster

754. Biomarkers and Drug Delivery Systems

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Topic: I.05. Biomarker and Drug Discovery

Support: Yonsei University Future-leading Research Initiative (Yonsei Challenge) of 2015 (2015-22-0137)

Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and Future Planning (2015R1C1A1A02036851)

Title: Efficacy and safety related factors after transient blood brain barrier opening by low intensity focused ultrasound

Authors: *J. SHIN[1,2], Y. NA[3], C. KONG[1], J. LEE[1,2], J. CHO[1], J. SEO[4], W. CHANG[1], J. CHANG[1,2];
[1]Dept. of Neurosurg., [2]Brain Korea 21 PLUS Project for Med. Sci. and Brain Res. Inst., Yonsei Univ. Col. of Med., Seoul, Korea, Republic of; [3]Dept. of Neurosurg., Catholic Kwandong Univ. Col. of Medicine, Intl. St Mary's Hosp., Incheon Metropolitan City, Korea, Republic of; [4]Dept. of Biomed. Engin., Yonsei Univ., Wonju, Korea, Republic of

Abstract: Introduction: For the treatment of neurodegenerative disorders and neuro-oncological disorders, various drug and chemotherapy agents have been developed and tried in clinical field. However, these drugs or chemotherapeutic agents showed limited efficacy because of blocking by the blood brain barrier (BBB). Recently, a local and selective drug delivery method using focused ultrasound (FUS), which was traditionally used for tissue destruction, was introduced for localized and transient opening of BBB. In this study, we tried to evaluate various parameter factors for optimized BBB opening condition in small animals. **Materials and Methods:** We have observed changes in BBB permeability of SD-rat during transcranial sonication with microbubble (MB) using low-intensity focused ultrasound. Moreover, to find the optimal sonication parameters for BBB-disruption, we examined various sonication factors: MB

injection type, MB type, pulse frequency, acoustic pressure, pulse repeated frequency and duty cycle. The animals were sacrificed and perfused 4h after FUS sonication. Brain tissues were obtained, sectioned and stained with hematoxylin and eosin (H&E) for histological examination **Results:** BBB opening at the sonication region was visualized using Evans blue (EB), which has a very large molecular weight of about 900Da and normally does not pass through the BBB. When BBB was disrupted, however, EB diffused through the barrier. Moreover, we were able to detect various amounts and forms of EB extravasation depending on the various sonication factors. Based on these results, we also demonstrated the optimal BBB-opening condition of the ultra-sonication for drug delivery. **Conclusion:** In this pre-clinical study, we demonstrated that FUS can facilitate optimized opening of BBB at the focal area. It is expected that this technique can be applied to targeted drug delivery into a localized brain area. However, further investigation regarding the limitation of molecular weight for transposition across the BBB, controlling of focus size and location, and optimal parameters for drug delivery according to the molecular weight is necessary before application to the clinical field.**Acknowledgement:** This study was supported by the grant from the Yonsei University Future-leading Research Initiative (Yonsei Challenge) of 2015 (2015-22-0137) and Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and Future Planning (2015R1C1A1A02036851).

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Poster

754. Biomarkers and Drug Delivery Systems

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Program#/Poster#: 754.22/NNN16

Topic: I.05. Biomarker and Drug Discovery

Support: Albion College Psychological Science

Albion College Foundation for Undergraduate Research, Scholarship, and Creative Activity

Title: Behavioral comparison of two methods of drug administration in the earthworm

Authors: B. A. JOHNSON, E. K. SEARS, *W. J. WILSON;
Psychological Sci., Albion Col., Albion, MI

Abstract: Earthworms are capable of Pavlovian and instrumental learning, offering an inexpensive and readily available alternative to mammalian or more exotic invertebrate species used in studies of memory. Drug studies in these soft-bodied organisms are made difficult by some uncertainty about methods of administration. Injection is possible; e.g., Watanabe et al. (2005) anesthetized worms for 10 min in 10% ethanol in order to inject protein synthesis inhibitors. A simpler approach that avoids anesthesia exists: worms can absorb drugs through their skin. We immersed worms in drug solutions, comparing time of immersion and concentration of solution as ways to manipulate dosage.Movement of *Lumbricus terrestris* was monitored in a running wheel in the dark before and after exposure to an aversive light. Worms received doses of caffeine by immersion in three different concentrations (9.15mM, 22.46mM, or 82.38mM) for an equivalent time (1 min), or in one concentration (9.15mM) for three different times (1, 3, or 9 min). The concentration-dependent doses caused different effects on the worms, with the low dose decreasing locomotion compared to either a high dose or water. The caffeine also decreased locomotion generally in the time-based doses, but there was no significant difference in locomotion between the doses. With both methods of administration, caffeine at lower doses decreased movement in response to the aversive light. We conclude that immersion in differing concentrations is a more reliable way to effect differing drug exposures than varying time of exposure. Analysis of drug within the worm after these manipulations is an obvious next step.

Disclosures: B.A. Johnson: None. **E.K. Sears:** None. **W.J. Wilson:** None.

Poster

754. Biomarkers and Drug Delivery Systems

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Program#/Poster#: 754.23/NNN17

Topic: I.05. Biomarker and Drug Discovery

Title: A correlation of serotonin 2A receptor occupancy and free fraction in rats.

Authors: J. THENTU, *G. BHYRAPUNENI, V. PALACHARLA, R. NIROGI;
Suven Life Sci., Hyderabad, India

Abstract: Second generation antipsychotics (atypical) affinity towards 5-HT_{2A} receptors could be the reason for reduced side effects. Aiming this receptor type has been the predominant target in drug discovery next to major D_2 receptor in schizophrenia treatment. Further, 5-HT_{2A} receptor antagonists have antidepressant and anti-anxiety properties and agonists have hallucinogenic effect. It is necessary to introduce a better parameter to predict the amount of 5-HT_{2A} receptors being interacted (occupied) with selective ligand concentration that shows desired

pharmacological actions. Supporting studies towards free drug hypothesis at different targets can be applied to bring a correlation between in-vivo 5-HT_{2A} receptor occupancy and in-vitro drug parameters (free fractions) at target site. Five standard compounds (quetiapine, eplivanserin, ketanserin, clozapine and olanzapine) were selected for in-vivo 5-HT_{2A} receptor occupancy assay using non-radiolabelled tracer (MDL 100907) in rats. Brain regional tracer concentration, drug exposures in brain and plasma from treated rats were analyzed by LC/MS-MS. Unbound fractions of compound in brain and plasma was estimated by HT-dialysis. Free forms of compound in plasma or brain are calculated from the product of in-vivo total plasma or brain drug concentrations and in-vitro free fractional value. Brain or plasma concentrations were normalized with their Ki before bringing a comparison. An association was inspected among in-vitro affinity constant (Ki); in-vitro free fractions of drug in plasma or brain; dose producing 50% occupancy at 5-HT_{2A} receptors (ED_{50}). The dose dependent % $5HT_{2A}$ receptor occupancy was found for all tested compounds which are consistent with in-vitro affinity profile (Ki, nM). Significant improvement in correlation was seen between free brain or plasma EC_{50} vs in-vitro Ki than with total compound concentration. Comparison between total brain and free brain concentration revealed the impact of permeability and efflux pumps on compound concentrations. Linear relation (R^2) between occupancy and free brain concentrations favors exact prediction of receptor occupancy.

Disclosures: **J. Thentu:** A. Employment/Salary (full or part-time): SUVEN LIFE SCIENCES LTD., HYDERABAD, INDIA. **G. Bhyrapuneni:** A. Employment/Salary (full or part-time): SUVEN LIFE SCIENCES LTD., HYDERABAD, INDIA. **V. Palacharla:** A. Employment/Salary (full or part-time): SUVEN LIFE SCIENCES LTD., HYDERABAD, INDIA. **R. Nirogi:** A. Employment/Salary (full or part-time): SUVEN LIFE SCIENCES LTD., HYDERABAD, INDIA.

Poster

754. Biomarkers and Drug Delivery Systems

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 754.24/NNN18

Topic: I.05. Biomarker and Drug Discovery

Support: MS Society Fast Forward

Title: A small molecule therapy for MS patients appears to override inhibitors of oligodendrogenesis to induce remyelination

Authors: ***S. H. NYE**, J. G. YARGER;
Discovery, ENDECE, LLC, Mequon, WI

Abstract: There is an unmet need for remyelinating therapies to treat multiple sclerosis (MS) patients. NDC-1308 is an analog of estradiol (E2) that harnesses the body's natural remyelinating system to drive oligodendrogenesis, a process resulting in mature, myelinating oligodendrocytes (OLs) that can repair damaged myelin sheaths. NDC-1308 was previously shown in oligodendrocyte progenitor cell (OPC) cultures to induce a 3-fold increase in OLs compared to vehicle. Structurally related estrogens, E2 and estriol, do not possess this activity. Side-by-side comparison of NDC-1308 and E2 activity, following chronic treatment in the cuprizone mouse model of demyelination, showed only NDC-1308 could significantly repair the myelin sheath (a 44% increase in hippocampus). NDC-1308 can apparently accomplish this by overriding inhibitors of oligodendrogenesis, such as Lingo-1. Here, we investigated how NDC-1308 has gained the biological activity to repair demyelinated axons, but lost the deleterious side-effects commonly associated with estrogens. While NDC-1308 and E2 are both ER agonists, we found the remyelinating activity of NDC-1308 can be traced back to its unique ability to significantly up-regulate key genes (OLIG2, DNER, MOG and MBP) for oligodendrogenesis. Real-time qPCR analysis showed these same genes are up-regulated 2-3 fold in human PBMCs treated for 12 hours with NDC-1308, suggesting they could serve as potential therapeutic biomarkers. Potential safety concerns for NDC-1308 were addressed. Estrogenicity was directly measured in a mouse uterotrophic assay since E2 treatment is known to cause a rapid and dramatic increase in uterine weight in this assay. Unlike E2, NDC-1308 was not found to be estrogenic. Further testing revealed that NDC-1308 is not mutagenic (Ames assay) and not genotoxic (micronucleus assay). The OPC pool remained intact after six weeks of chronic NDC-1308 treatment, demonstrating that it can serve as a renewable source for sustaining oligodendrogenesis. In conclusion, NDC-1308 is a potential first-in-class remyelinating therapy that possesses many key qualities needed to effectively treat secondary progress (SPMS) and relapsing-remitting (RRMS) MS patients.

Disclosures: **S.H. Nye:** A. Employment/Salary (full or part-time): ENDECE, LLC. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ENDECE, LLC. **J.G. Yarger:** A. Employment/Salary (full or part-time): ENDECE, LLC. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ENDECE, LLC.

Poster

754. Biomarkers and Drug Delivery Systems

Location: Halls B-H

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Topic: I.05. Biomarker and Drug Discovery

Support: Neuropathology Training Grant 5T32NS007098-33

Title: RNA-based agonists and antagonists (aptamers) for FGFR3 to modulate activation of astrocytes in a glial scar model

Authors: *N. KAMATKAR[1], M. LEVY[2], J. HÉBERT[3];
[1]Albert Einstein Col. of Med., Bronx, NY; [2]Biochem., [3]Neuroscience, Genet., Albert Einstein Col. of Med., Bronx, New York City, NY

Abstract: Astrogliosis is the process by which astrocytes become activated after brain injury. Astrocyte activation takes place after virtually any insult to the central nervous system including trauma, infection, and cell death. It is a complex and graded process that relies on multiple cellular signals for not only astrocyte activation but also to inactivate astrocytes. In severe instances, astrocyte activation can result in the formation of a glial scar that can be both beneficial in terms of restricting inflammation and detrimental since it inhibits axon regeneration. Recently, my lab has discovered that the fibroblast growth factor (FGF) signaling pathway plays a critical role in the inhibition of astrocyte activation in the normal and injured brain. Moreover, we have also demonstrated that both gain-of-function and loss-of-function studies of FGF signaling result in a decrease in glial scar size. The FGFs are a family of secreted proteins used for angiogenesis, wound healing, and multiple other roles in the developing organism and also play an important role in numerous pathological processes. To date, there are limited options for people who suffer from FGF signaling related diseases since there are no specific agonists or antagonists for the receptors of FGF signaling. Here, I plan to develop novel molecular tools specific for all three fibroblast growth factor receptors (FGFRs) expressed in the brain (FGFR1,2,3) and test them as therapeutics in glial scar formation. To achieve this goal, I will develop aptamers, nucleic acid ligands that have the potential to be specific antagonists and agonists to modulate signaling through the FGFRs. Importantly, I have already identified a number of nuclease stabilized ligands to FGFR3. One aptamer specifically, NK01, demonstrates high affinity for FGFR3 and inhibits FGF2 from binding FGFR3. Perhaps more interestingly, another aptamer, C20, stimulates signaling from FGFR3 when presented to the cells as a multimer. Ultimately, I will develop a suite of novel tools to interrogate FGF signaling at the level of the receptor that can be used as possible therapeutics, particularly in glial scar formation.

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Poster

754. Biomarkers and Drug Delivery Systems

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Topic: I.05. Biomarker and Drug Discovery

Support: National Multiple Sclerosis Society CA1069-A-7

Title: Does cognitive fatigue correlate with brain iron deposition in basal ganglia in Multiple Sclerosis?

Authors: S. WOOD[1], E. DOBRYAKOVA[2], Z. JIANG[3], E. HAVRILLA[4], *B. YAO[1,5]; [1]Neuroimaging Ctr., [2]Traumatic Brain Injury Lab., [3]Human Performance Engin. Lab., Kessler Fndn., West Orange, NJ; [4]Montclair State Univ., Montclair, NJ; [5]Dept. of Physical Med. & Rehabil., Rutgers, The State Univ. of New Jersey, Newark, NJ

Abstract: BACKGROUND: Fatigue, defined as an overwhelming feeling of lack of both mental and physical energy, has been reported in over 90% of individuals with multiple sclerosis (MS). Studies have shown basal ganglia structures play a central role in fatigue. Meanwhile, abnormal iron deposition has been observed in the deep gray matter structures including basal ganglia in MS. In this study, we aimed to examine the correlation between brain iron concentration indicated by susceptibility contrast imaging and the severity of fatigue in MS. **METHODS:** Six clinically definite MS patients participated in this study. *MRI:* A 3D multi-echo gradient-echo acquisition was performed on a 3T scanner. Quantitative R_2^* maps were derived from exponential fitting over five echo data. The susceptibility maps (QSM) were calculated using the LSQR algorithm based on the unwrapped phase maps. Six regions of interest (ROIs) including substantia nigra (SN), red nucleus (RN), globus pallidus (GP), putamen (PU), caudate nucleus (CN), and thalamus (TH) were manually drawn on the magnitude images. R_2^*, and Frequency, and QSM values were averaged in each ROI, respectively, and then averaged across all the subjects in the group. *Fatigue measures:* Each individual were administrated a Fatigue Severity Scale (FSS) test and a Modified Fatigue Impact Scale (MFIS) test to measure their fatigue levels. The FSS scores and total MFIS scores with its subcategories (Physical, Cognitive, Psychosocial) subscales from each individual were correlated with R_2^*, Frequency shift and QSM values in all ROIs. **RESULTS:** Significant positive correlations ($p < 0.05$) between Frequency and FSS Total ($r = 0.79$), MFIS Total ($r = 0.97$), MFIS Physical subscale ($r = 0.94$) and MFIS Psychosocial subscale ($r = 0.86$) are found in CN. QSM also correlates with MFIS Total ($r = 0.76$) and MFIS Physical subscales ($r = 0.82$) significantly. No significant consistent positive correlations in the other ROIs are found. No significant correlations between R_2^* and all fatigue measures are observed. **DISCUSSION:** Basal ganglia is of particular interest as its damage is often associated with clinical disorders including MS. Our results show a promising correlation between iron-related MRI indices with fatigue scores, indicating the severity of fatigue may correspond to iron accumulation in CN. This result is consistent with our previous findings that iron deposition is found to be higher in basal ganglia in MS patients comparing to healthy control individuals. These findings are of particular interesting to understanding the fatigue mechanisms, which may lead to an effective treatment on reducing clinical symptoms in MS patients.

Disclosures: **S. Wood:** None. **E. Dobryakova:** None. **Z. Jiang:** None. **E. Havrilla:** None. **B. Yao:** None.

Poster

754. Biomarkers and Drug Delivery Systems

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Program#/Poster#: 754.27/NNN21

Topic: I.05. Biomarker and Drug Discovery

Title: Minocycline-loaded nanoparticles for reducing tissue reactions after neural electrode implantation

Authors: *A. D. HOLMKVIST[1,2], J. AGORELIUS[1], M. FORNI[1], C. ERIKSSON LINSMEIER[1], U. J. NILSSON[2], J. SCHOUENBORG[1];
[1]Neuronano Res. Ctr., Lund, Sweden; [2]Dept. of Chem., Ctr. for Analysis and Synthesis, Lund, Sweden

Abstract: Implantable neural interfaces are important tools for the investigation of neurophysiological systems and have several clinical applications. However, the biocompatibility and chronic functional stability of these implanted interfaces are far from optimized. It is well known that the implant subsequently elicits both acute and chronic reactions in the surrounding tissue, which leads to the formation of an insulating glial scar and electrode failure.The objective of this work is to investigate the use of biodegradable nanoparticles for sustained delivery of minocycline, a neuro-protective and anti-inflammatory drug, locally in the brain after electrode implantation. Minocycline-loaded PLGA nanoparticles were prepared by a single oil-in-water based preparation technique and characterized for size (~220 nm), drug content (1.1%) and drug release. The *in vitro* release profile showed drug release over 30 days, which provides a good match to the normal time course of brain tissue responses associated with electrode implantations.Stainless steel needles were embedded with gelatin containing the minocycline-loaded nanoparticles and implanted into cortex of CX3CR1-GFP knock-in mice with fluorescently labeled microglial cells. The tissue response after implantation was studied. The results presented here show the importance of controlling the local milieu in the near vicinity of the electrode for mastering tissue reactions and provides an additional step in designing fully biocompatible neural interfaces.

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Poster

754. Biomarkers and Drug Delivery Systems

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Program#/Poster#: 754.28/NNN22

Topic: I.05. Biomarker and Drug Discovery

Title: A novel approach to ischemic stroke biomarker discovery

Authors: *A. A. DAUBENSPECK[1], D. R. COOL[2], B. R. LUDWIG[3], J. E. OLSON[1]; [1]Emergency Med., Wright State Univ., Kettering, OH; [2]Pharmacol. and Toxicology, Wright State Univ., Dayton, OH; [3]Neurol., Premier Hlth. Clin. Neurosci. Institute, Wright State Univ. Boonshoft Sch. of Med., Dayton, OH

Abstract: Ischemic stroke (IS), a leading cause of death and disability worldwide, requires rapid and accurate diagnosis. However, IS diagnosis in the emergency setting is currently limited to clinical examination of symptoms and low-sensitivity CT imaging. No direct, objective, point of care test is currently available that can diagnose IS in the time frame needed to institute appropriate therapy. This need could be met by measuring levels of protein biomarkers in circulating blood. Several proteins have shown promise in pre-clinical trials; however, because of the heterogeneity of the disease, these singular biomarker candidates have shown inadequate sensitivity and specificity in clinical studies. Therefore, a combination of protein biomarkers associated with brain ischemia is thought to be necessary for emergency IS diagnosis.
We take a novel approach to ischemic stroke biomarker discovery. Combining an endovascular procedure with proteomic analysis we quantitatively catalogue changes in the abundance of all serum proteins present in blood from the penumbra of stroke patients. Patients diagnosed with ischemic stroke who undergo mechanical thrombectomy are included in this IRB-approved study. During the thrombectomy procedure a micro-catheter is used to withdraw arterial blood samples from the general circulation (peripheral) and from the region of ischemic brain area (penumbral) prior to clot removal. For each blood sample, the serum is depleted of albumin, salts, lipids, and nucleic acids. Then an aliquot of each blood sample containing 25 μg protein is labeled with a different fluorescent dye. Samples are combined and then separated by 2-dimensional gel electrophoresis (2D-DIGE) first by isoelectric focusing (pH=3 to pH=10), then by SDS-PAGE. The resulting gel images are analyzed using Image J software for changes in relative protein abundance as determined by their respective fluorescence intensities.
Gels from 4 initial patients showed over 50 individual protein spots. Twenty-five of these spots were chosen for analysis in this preliminary study. The ratio of fluorescence intensities between peripheral and penumbral samples was used for univariate analysis and pair-wise statistics. Of the 25 protein spots analyzed, 5 proteins show a significant change of 20% or more and thus may constitute an initial test panel of protein stroke biomarkers. Proteins with differential expression in the penumbral blood are likely to originate from ischemic brain tissue and could be considered

biomarker candidates. Furthermore, the changes in multiple protein abundances found in penumbral blood support our approach to novel biomarker discovery.

Disclosures: **A.A. Daubenspeck:** None. **D.R. Cool:** None. **B.R. Ludwig:** None. **J.E. Olson:** None.

Poster

754. Biomarkers and Drug Delivery Systems

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Program#/Poster#: 754.29/NNN23

Topic: I.05. Biomarker and Drug Discovery

Title: Evaluation of brain pharmacokinetic properties in awake animals: from rodents to non-human primates

Authors: ***M. VAN GAALEN**[1], G. FLIK[2], J. H. A. FOLGERING[2], A. RASSOULPOUR[3], M. CHOI[4], R. STRATFORD[4], T. I. F. H. CREMERS[5];
[1]Encepharm, Goettingen, Germany; [2]Brains On-Line, Groningen, Netherlands; [3]Brains On-Line, San Francisco, CA; [4]Duquesne Univ., Pittsburgh, KS; [5]Dept. of Pharmaceut. Analysis, Univ. of Groningen, Groningen, Netherlands

Abstract: Of the clinical candidates that enter phase 1, approximately one out of ten is approved by FDA. This low success rate is very concerning for drug developers, regulators and patients. Unacceptable pharmacokinetic properties is in part the reason for discontinuation of clinical development. An additional hurdle comes in play for neurological and psychiatric indications: the blood brain barrier. Clinical candidates need to be selected on the property to reach the target, and therefore, adequate free brain concentrations need to be reached that can induce the desired pharmacodynamic effects. This can be directly measured in awake animals by using Metaquant microdialysis in various species. In the present study, we compared the absolute brain concentrations as well as CSF concentrations of dextroamphetamine and its pharmacodynamics effect in rats and non-humans primates (NHPs). Wistar rats and cynomolgus monkeys received dextroamphetamine. The concentration of dextroamphetamine was measured bilaterally in the prefrontal cortex (PFC) and striatum (Str) up to 6 hours post dosing. A separate group of animals receiving the same dose had an indwelling catheter surgically placed into the cisterna magna to support cerebrospinal fluid (CSF) sampling for up to 4 hours post-dosing. Compound concentrations in plasma were measured via venous sampling. In monkeys, ECF and CSF dextroamphetamine concentrations were similar to each other and to unbound plasma drug concentrations across the time course and brain regions, supporting rapid equilibration of compound throughout the monkey CNS. In rats, ECF and CSF concentrations were similar, but

appeared to be 1.5 to 2 times greater than plasma unbound concentrations over the sample time course. Equilibration was also achieved by the first sample time point (30 minutes). These results suggest facile movement of dextroamphetamine across the blood-brain barrier of both species, possibly also the choroid plexus, and rapid equilibration within the CNS. Observation of a net uptake of dextroamphetamine into rat CNS, but not monkeys, may indicate an important species difference in the absorption of this drug into the brain. This study confirms that pharmacokinetic and pharmacodynamics properties may differ between species. Furthermore, it indicates that cross species evaluation is valuable to select clinical candidates and their predictive efficacy dose.

Disclosures: **M. Van Gaalen:** None. **G. Flik:** None. **J.H.A. Folgering:** None. **A. Rassoulpour:** None. **M. Choi:** None. **R. Stratford:** None. **T.I.F.H. Cremers:** None.

Poster

754. Biomarkers and Drug Delivery Systems

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 754.30/NNN24

Topic: I.05. Biomarker and Drug Discovery

Support: Arizona Alzheimers Consortium

Title: Ultrasound-mediated delivery across the blood brain barrier.

Authors: *E. G. FERNANDEZ[1], M. VALDEZ[2], M. ROMANOWSKI[2], T. MATSUNAGA[3], R. WITTE[3], T. P. TROUARD[2];
[1]Neurosci. and Cognitive Sci., [2]Biomed. Engin., [3]Med. Imaging, The Univ. of Arizona, Tucson, AZ

Abstract: Treatment of neurological disorders is often hampered by the inability of therapeutics to cross the blood-brain barrier (BBB). Over the last several years, novel techniques have been developed that use focused ultrasound (FUS) in combination with microbubble (μB) contrast agents to temporarily open up the BBB and allow therapeutics into the brain. Foundational studies have shown BBB-opening is clearly visible via contrast-enhanced MRI. In this work, MRI and fluorescence microscopy were used to analyze the distribution of different sized molecules following BBB-opening in mice. BBB-opening was carried out in anesthetized mice using a custom-made cradle and FUS system. Following a 0.2 μL/g IV injection of μBs and an IP injection of Gd-DTPA, FUS was applied for 2 min (10 ms pulse at 1 Hz). Mice were then given another IV injection of 3, 70, and 500 kD dextran (1 mg each) with a different fluorescent labels. Mice were imaged using T1-weighted MRI on a 7T Bruker BioSpec MRI system and then

perfused with PBS and paraformaldehyde. Brains were cryosectioned and imaged with an Olympus MVX10 fluorescence microscope. Fluorescent signal was quantified by determining the volume of brain exhibiting fluorescent signal greater than the background autofluorescence. Safety of the procedure was evaluated by opening the BBB using different levels of FUS pressures (72, 120 and 214 kPa peak negative pressures). These brains were H&E-stained and imaged to quantify microbleeds and neuronal damage. MRI and fluorescence microscopy images following BBB-opening showed a strong co-localization of MRI signal enhancement with the fluorescent dextrans. The volume of brain with Gd-enhancement and dye increased with pressure and decreased with dextran size. The smaller molecules showed a diffuse pattern of enhancement while the larger molecules appeared in punctate patterns. Significant opening of the BBB with no visible damage was observed at120 kPa. Higher pressures showed damage, and lower pressures showed little to no BBB-opening. These results demonstrate FUS can safely open the BBB and allow molecules of various sizes to enter the brain with differential distribution. While the BBB-opening technique is intended to increase drug delivery to the brain for neurological disorders (e.g. Alzheimer's and Parkinson's), these imaging experiments could also be utilized to evaluate the loss of BBB integrity caused by other pathologies such as brain tumors, TBI, and viral infections.

Disclosures: E.G. Fernandez: None. **M. Valdez:** None. **M. Romanowski:** None. **T. Matsunaga:** None. **R. Witte:** None. **T.P. Trouard:** None.

Poster

754. Biomarkers and Drug Delivery Systems

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 754.31/NNN25

Topic: I.05. Biomarker and Drug Discovery

Support: NIH 1 RO1 MH104147-01

Title: Novel multi-step urmc-099 neuroprotective, antiretroviral and autophagy-associated cell responses to combat hiv-1 infection

Authors: D. GNANADHAS[1], P. DASH[1], Z. LIN[1], J. M. PUCCINI[3], H. A. GELBARD[3], *H. E. GENDELMAN[2], S. GORANTLA[1];
[1]Pharmacol. and Exptl. Neurosci., [2]Prof & Chair Dept Pharmacol, Univ. of Nebraska Med. Ctr., Omaha, NE; [3]Ctr. for Neural Develop. and Dis., Univ. of Rochester Med. Ctr., Rochester, NY

Abstract: Originally developed as an adjunctive neuroprotective and anti-inflammatory agent for HIV-1 associated neurocognitive disorders (HAND), URMC-099, a mixed lineage kinase-3

inhibitor also modulates long-acting nanoformulated antiretroviral therapy (nanoART) biodistribution, pharmacokinetic and pharmacodynamics (PD) responses by sequestering ART particles in early, late and recycling endosomes. However, obstacles in the bench to bedside translation of nanoART remain including optimization of drug retention, ease of administration and toxicity for the nervous and reticuloendothelial systems. We demonstrated that decreased phosphorylation of JNK and changes in the trafficking of nanoART lead to reduced viral loads and increased CD4+ T lymphocyte in HIV-1 infected humanized mice. Since, JNK regulates mTORC1 and mTORC1 controls vesicular trafficking through Transcription Factor EB (TFEB), we hypothesized that URMC-099 can affect vesicular trafficking through TFEB. To test this notion we used human monocyte-derived macrophages (MDM) pretreated with nanoformulated atazanavir (nanoATV) and infected the cells with the HIV-1 Ada viral strain with or without URMC-099. Increased nuclear translocation of TFEB was observed by inhibition mTORC1 following URMC-099 treatments. As TFEB regulates autophagy, we now show that increases in LC3B, BECN1 and decreased P62 by Western blot and qRT-PCR assays. Increased numbers of autophagosomes were seen following URMC-099 treatment that were decreased by inhibition of autophagy. Increased mitochondrial activity also followed URMC-099 treatment. NanoATV was increased in URMC-099 treated MDM and found specifically localized in LC3B autophagosomes. As autophagosomes are involved in HIV-1 assembly and maturation, increase in autophagy with retention of nanoATV in autophagosomes by URMC-099 facilitated viral clearance in MDM exposed to virus. Together, URMC-099 improves cell health, retains nanoART and facilitates HIV-1 clearance through induction of autophagy. As URMC-099 is a brain penetrant and have shown neuro protective activity, this finding opens up novel therapeutic strategies for URMC-099 for neuroAIDS.

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Poster

755. Computational Tools: Outcomes and Evaluations

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 755.01/NNN26

Topic: I.06. Computation, Modeling, and Simulation

Support: The Scientific and Technological Research Council of Turkey (TÜBİTAK), Grant No: EEEAG-115E257

Bogazici University BAP Grants #10XD3

Bogazici University Life Sciences and Technologies Research Center #09K120520

Title: Bioinspired Neuroprosthetic Design Environment (BNDE): A platform for creating hybrid biological/in silico neural networks for motor neuroprosthetic control

Authors: *M. KOCATURK[1], S. TOPKARAOGLU[2], K. N. ISIK[2], S. KOCATURK[1], F. Y. YAMANER[2], T. BAYKAS[2], A. GUVENIS[3], H. O. GULCUR[3], R. CANBEYLI[4];
[1]Dept. of Biomed. Engin., [2]Dept. of Electrical and Electronics Engin., Istanbul Medipol Univ., Istanbul, Turkey; [3]Inst. of Biomed. Engin., [4]Dept. of Psychology, Bogazici Univ., Istanbul, Turkey

Abstract: Motor neuroprostheses aim to restore functions lost to disease or injury by directly communicating with the brain tissue. In the design of neuroprosthetic controllers, an input-output mathematical model, generally based on a filter (e.g. Kalman filter) is used conventionally to convert firing rates of recorded neurons into control commands for prosthetic actuators. Based on an input-output model or transform, information processing principles of these systems are fundamentally different from those of natural neural circuits. In this work, we present the Bioinspired Neuroprosthetic Design Environment (BNDE) which enables the development of neuroprosthetic controllers from a novel, more biologically plausible design perspective. The BNDE has been implemented to create neuroprosthetic controllers which are based on spiking neural networks (SNNs). The SNN-based controller in the present architecture receives simulated synaptic inputs from extracellularly recorded neurons and forms a hybrid biological/in silico neural network with the neuronal circuits of the user's brain.
We developed a new, adaptive neuroprosthetic controller using real-time closed-loop simulations to test the BNDE prior to its use in in vivo experiments. The controller consists of two model (in silico) medium spiny neurons, each of which receives simulated synaptic inputs from recorded motor cortical neurons. In the closed-loop simulations, the recordings from the cortical neurons were imitated using an external, hardware-based neural signal synthesizer. By implementing a dopamine-dependent spike timing-dependent plasticity (STDP) rule, the controller achieves perfect target reach accuracy for a two-target reaching task in one-dimensional space.
The BNDE, developed around a PC equipped with an Intel Core i7-4790K CPU and RTAI 5.0 (Real-time Application Interface for Linux), is capable of real-time simulation of 180 Izhikevich neurons receiving virtual synaptic inputs from external spike data resources through 32 data acquisition channels.
In the BNDE, the spike events recorded from real neurons are directly forwarded to the SNN-based neuroprosthetic controller. Since there is no spike binning process in the present system in contrast to the conventional neuroprosthetic systems, the information encoded by the timing of the spikes are also used by the controller. As spike timing plays a critical role in neuroplasticity and neuroprosthetic learning, the SNN-based neuroprosthetic controllers updating their parameters by simulating mechanisms of STDP may have superior adaptation performance than existing firing rate-based neuroprosthetic systems.

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Poster

755. Computational Tools: Outcomes and Evaluations

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 755.02/NNN27

Topic: I.06. Computation, Modeling, and Simulation

Title: Application of the k-nearest neighbor algorithm and multiple look integration to fuse information for intelligent decision making processes

Authors: *H. C. YUAN[1], M. V. CHAO[2];
[1]Independent Lab., San Marino, CA; [2]Independent Lab., Palos Verdes, CA

Abstract: The k-Nearest Neighbor (kNN)algorithm has been used for a wide spectrum of classification problems as predicting heart attack based on patient diet and clinical measurements, bank customer profiling based credit ratings and income, and text classification based on training documents of known categories. Features used for these classification applications are rather static or do not change rapidly with time. The timeframes could be in weeks for patient diet measurements, in months for credit card usage information, or in hours for text classification training documents. In contrast, intelligent decision making processes may require rapid information updates over multiple looks as fast as 1 update per second, or faster. An extension of multiple look in machine learning to how the brain might integrate and fuse dynamic information could be posed to answer the question: “Is it a bird or a plane?” Multiple look could be a natural process in the brain. This leads to consideration of the multiple look processes to handle noisy, scintillating data, or imperfect data, then using a simple instance-based classifer as kNN to process a classifer result. In aerospace applications, radar kinematic data as distance, altitude, velocity, and acceleration can be used to classify different types of airborne vehicles as: helicopters, commercial passenger jets, crop dusters, or fighter jets. Prototype vectors of typical, measured, or theoretical kinematic data of these different types of airborne vehicles could be stored in a computer database. A radar sensor collecting and measuring kinematic information would form a kinematic data vector and the k-nearest neighboring known prototype vectors would be polled for a majority and the resulting airborne vehicle classified according to the majority of k-nearest prototype vectors. The update rate for radar kinematic being rapid could provide options to integrate multiple looks of input kinematic data vectors over a very short period of time to overcome system noise, scintillation, errors in the radar sensor, or errors in the kinematic measurement. In similar contrast, high range resolution feature data vectors of aircraft were combined in a multiple look integration [Kahler and Blasch, 2008] to distinguish between multiple moving targets in clutter. Similarly, the brain could be rapidly integrating multiple looks of data or sensor inputs in order to make timely, intelligent decisions. This poster examines the use of multiple look integration with the k-Nearest-Neighbor

algorithm as a computational model for intelligent decision making in machine learning processes and artificial intelligence.

Disclosures: H.C. Yuan: None. **M.V. Chao:** None.

Poster

755. Computational Tools: Outcomes and Evaluations

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 755.03/NNN28

Topic: I.06. Computation, Modeling, and Simulation

Title: The contribution of graph-derived features to a cloud-based distributed neuroscience publication classification pipeline

Authors: *K. H. AMBERT[1], K. S. MUPPALLA[2];
[1]Big Data Solutions, Intel, Tigard, OR; [2]Big Data Solutions, Intel, Hillsboro, OR

Abstract: To help keep pace with the publication rate in the biomedical literature, there is a need for machine-driven approaches to automated and semi-automated knowledgebase curation. Such techniques have been shown to be particularly useful for the identification of publications containing information of interest for neuroinformaticians who are curating knowledge bases for informing multi-level models of brain function. Although using such systems to classify and prioritize documents for curation workflows is not a new idea, there continues to be developments in scalable text analytic and graphical machine learning methodology that uncover the possibilities of new approaches to this problem. Here, we describe the implementation of a scalable cloud-based document classification pipeline for identifying publications in the neurosciences that contain novel information for a neuron morphophysiology knowledgebase. Our series of experiments describe the textual and graph-derived feature selection and machine learning algorithm optimization experiments that led to the best-performing system. Further, we describe the open source cloud architecture used to deploy this machine learning solution, and how it can be incorporated into a neuroscience research workflow.

Disclosures: K.H. Ambert: A. Employment/Salary (full or part-time): Intel. **K.S. Muppalla:** A. Employment/Salary (full or part-time): Intel.

Poster

755. Computational Tools: Outcomes and Evaluations

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 755.04/NNN29

Topic: I.06. Computation, Modeling, and Simulation

Support: FCT UID/MAT/00297/2013

ANR-14-CE13-0030-01 PHYSIOBS

Title: Algorithmic design of a therapeutically- and energy-efficient closed-loop deep brain stimulation system: a computational study

Authors: *S. KARAMINTZIOU[1], A. L. CUSTÓDIO[2], B. PIALLAT[3,4], M. POLOSAN[3,5], S. CHABARDÈS[3,5,4], P. G. STATHIS[6], G. A. TAGARIS[7], D. E. SAKAS[8], G. E. POLYCHRONAKI[1], G. L. TSIROGIANNIS[1], O. DAVID[3,4], K. S. NIKITA[1];
[1]Natl. Tech. Univ. of Athens, Athens, Greece; [2]Dept. of Mathematics, FCT-UNL-CMA, Lisbon, Portugal; [3]Grenoble Inst. of Neurosciences (INSERM-U836), Grenoble, France; [4]Brain Function and Neuromodulation, Joseph Fourier Univ., Grenoble, France; [5]Dept. of Psychiatry, Univ. Hosp. of Grenoble, Grenoble, France; [6]Dept. of Neurol., Mediterraneo Hosp., Athens, Greece; [7]Dept. of Neurol., 'G. Gennimatas' Gen. Hosp. of Athens, Athens, Greece; [8]Dept. of Neurosurg., 'Evangelismos' Gen. Hosp., Athens, Greece

Abstract: *Objective.* We elaborate on the algorithmic aspects of a closed-loop subthalamic nucleus (STN) *deep brain stimulation* (DBS) system for advanced Parkinson's disease (PD) and treatment-refractory obsessive-compulsive disorder (OCD), ensuring optimal performance in terms of both efficiency and selectivity of stimulation, as well as in terms of computational speed. *Methods.* Relying upon a series of methods robust to the presence of measurement noise, we first assess the presence of significant nonlinear coupling between beta and high-frequency subthalamic neuronal activity, as a biomarker for feedback control in the proposed closed-loop neuromodulation scheme. We then present a *model-based* strategy through which optimal and individually tailored parameters of stimulation for desynchronizing *control* of neuronal activity are being identified. Simulations are being performed utilizing microelectrode recordings (MERs) acquired during STN-DBS surgical interventions for PD and OCD. *Results.* Cross-frequency coupling proves to be a potentially appropriate biomarker for feedback control in case of PD, but may display subject-specific applicability in case of OCD. We demonstrate the ability of the presented modeling approach to identify, at a relatively low computational cost, personalized stimulation settings potentially associated with a significantly higher efficiency and selectivity compared with stimulation settings determined during post-operative clinical management of patients with PD and treatment-refractory OCD. *Conclusion.* Together, our data provide strong evidence for the applicability of computational neurostimulation to the

development of personalized stimulation protocols for the treatment of movement and neuropsychiatric disorders.

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Poster

755. Computational Tools: Outcomes and Evaluations

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 755.05/NNN30

Topic: I.06. Computation, Modeling, and Simulation

Title: Modeling the impact of time-delay effects on the memory capacity, cognition capability and energy efficiency of neuronal structures

Authors: *Y. G. TIRAT-GEFEN;
Castel Res. Inc., Fairfax, VA

Abstract: This study discusses a mathematical model incorporating time delay effects in large biological neuronal nets. We use mathematical techniques, such as perturbation theory and linearization by parts, to handle the highly nonlinear equations describing biological neural nets in the presence of large propagation delays. We consider the possibility that these delays can be modulated, instead of assuming time invariant delays. We consider time-delay effects due to other effects such as gene expression, which may be relevant on how stimulus can be stored as a long term memory. We investigate the impact of signal propagation delays on increasing the cognitive capacity of biological neuronal nets and their complexity. Our model considers the interplay between energy efficiency, partitioning of cognitive and sensory functions in large neuronal structures, and time delays. We found that the presence of time-delays in neuronal nets actually increases the number of available states to represent knowledge and improve the cognitive capability of neuronal structures. Another interesting effect if the apparent impossibility to determine both the neuronal state values and their derivatives in time with precision simultaneously. This implies a trade-off between increased cognition capability when time delays are present, and decreased state observability. We conclude with an analysis on how the emergence of large time delays and their impact on energy consumption in animals may have influenced the evolution of the human brain.

Disclosures: **Y.G. Tirat-Gefen:** None.

Poster

755. Computational Tools: Outcomes and Evaluations

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 755.06/NNN31

Topic: I.06. Computation, Modeling, and Simulation

Support: NIH Grant R01MH100820

Title: Measuring direction of synaptic transmission via cross frequency coupling

Authors: *B. NANDI[1], B. KOCSIS[2], M. DING[3];
[1]Biomed. Engin., Univ. of Florida, Gainesville, Gainesville, FL; [2]Harvard Med. Sch., Boston, MA; [3]Univ. of Florida, Gainesville, FL

Abstract: Cross Frequency Coupling (CFC) assesses the relationship between oscillatory activities in different frequency bands. The frequently applied phase-amplitude CFC estimates the statistical dependence between the phase of a low frequency oscillation and the amplitude of a high frequency oscillation and provides an index of effective integration of neural activities across different spatial and temporal scales. To date, CFC analysis has been mainly applied in a univariate fashion, namely, the high frequency amplitude and the low frequency phase are obtained from the same signal. In this work we considered phase-amplitude CFC between two different signals representing two different brain areas or neuronal ensembles. Assuming that the high frequency oscillatory amplitude reflects population spiking, therefore the output activity of a neuronal ensemble, and that the lower frequency oscillation reflects dendritic processing, therefore the input activity of a neuronal ensemble, we hypothesized that cross-area phase-amplitude CFC may be used to infer the direction and strength of synaptic transmission. We tested our hypothesis by analyzing laminar recordings from the hippocampus of anesthetized rats during theta rhythm elicited by brainstem stimulation. The 16-channel multi-electrode (100um separation between contacts) spanned CA1, dentate gyrus (DG), and hilar regions. The layers were identified by perforant path evoked potentials. We found that (1) the amplitude of gamma oscillations in DG was significantly coupled to the phase of theta oscillations in CA1 and (2) the amplitude of gamma oscillations in CA1 was not significantly coupled to the phase of theta oscillations in DG. These results, suggesting a unidirectional synaptic transmission from DG to CA1, are consistent with the prediction of the trisynaptic pathway linking DG to CA1 (DG to CA3 to CA1) as well as Granger causality analysis of the same data.

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Poster

755. Computational Tools: Outcomes and Evaluations

Location: Halls B-H

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Program#/Poster#: 755.07/NNN32

Topic: I.06. Computation, Modeling, and Simulation

Title: Performance driven neural network model representation: a relational approach

Authors: *S. L. GRATIY, A. ARKHIPOV, D. FENG, N. GOUWENS, N. CAIN, R. IYER, M. BUICE, S. MIHALAS, C. ANASTASSIOU;
Allen Inst. for Brain Sci., Seattle, WA

Abstract: The development of large-scale network simulations of brain circuits strongly benefits from having a standardized data schema for representing the parameters of a network and of individual neurons. A standardized data schema creates a common interface between modeling tools and allows for the splitting of the network modeling workflow into separate consecutive steps: building, simulation, and analysis/visualization. Furthermore, standardization is crucial for model sharing and reuse, as well as collaboration between scientists using a diverse set of computational tools.
Based on the needs of our research in the directions of biophysically detailed, point-neuron, and population-based network modeling, we have identified a number of essential requirements for network model representation. These are computational efficiency, scalability to large model size, simplicity (minimal need for external software tools), generality (allow for different levels of model detail), modularity (altering model components without affecting other aspects), human readability, and minimization of redundancy. Here we present progress in developing a network representation reflecting these principles.
We utilized the relational (i.e., table-based) approach for representing parameters of network entities that are linked by keys. We have identified several network entities each of which are represented within individual tables, e.g. cells, cell models, connection types, connections, cell morphology and cell intrinsic electrophysiology properties. The rows in tables contain the instances of each entity and the columns contain their attributes. Most attributes in tables may be viewed as optional allowing for greater flexibility of the data schema. Similarly, the number of entities described by individual tables is flexible and accommodates a specific network's level of detail. The data is stored on disk using either CSV or hdf5 file formats and is loaded into the memory as pandas data frames and numpy arrays, respectively. Concomitantly, we are working with the Blue Brain Project to arrive at an overall standardized network representation, which would meet joint modeling needs.

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Poster

755. Computational Tools: Outcomes and Evaluations

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 755.08/NNN33

Topic: I.06. Computation, Modeling, and Simulation

Support: France Parkinson

Carnot/EuroTalents

Title: Link between the infrared light power emitted by an implantable medical device and thermal elevation induced locally

Authors: *C. MORO[1], J. MOLET[1], V. AUBOIROUX[1], F. REINHART[1], S. RENAULT[1], C. CHABROL[1], N. TORRES-MARTINEZ[1], J. MITROFANIS[2], A.-L. BENABID[1];
[1]Cea-Grenoble, Leti-Clinatec, Grenoble, France; [2]Anat. department, Univ. of Sydney, Sydney, Australia

Abstract: Rationale: Several studies have highlighted the therapeutic potential of low-intensity light (near infrared) on various pathologies and neurodegenerative diseases such as Parkinson's disease. This new therapy has led to the development of novel implanted optical fiber devices. These devices may generate heat, that would lead to an increase tissue temperature and subsequent damage. In this study, we present a high spatial resolution, high precision characterization of the thermal effects of active, implanted optical fiber devices in order to assess their safety. We used high resolution MR-thermometry that allows quantification of thermal variations in the animal brain, without any bias such as induced by a thermocouple. **Methods:** We tested a device specifically designed for low-light intra-cerebral laser therapy at 670 nm, with optical fibers designed for the delivery of light in deep regions of the rat and monkey brain. We measured temperature at several light powers at the fiber tip. Total amount of light, instantaneous power emission, illumination length and schedule and wavelength were varied. Using proton resonance frequency shift (PRFS)-based MR-thermometry (resonance frequency dependence to biological tissue temperature), high resolution (0.3 x 0.3 x 0.1 mm^3) thermal maps were acquired during in vivo illumination in rat and monkey. Multi-slice MR acquisitions were performed using a 4.7 T Bruker imager. **Results:** We evaluated the temperature increase induced by varying optical powers applied at the end of the fiber. The objective was to determine optical powers that do not lead to a temperature increase above 2 °C. Preliminary data in rats showed that a 15 mW optical power did not cause a temperature increase above 2°C and mid-height energy was observed at a radius of about 1 mm, depending on the implanted anatomical structure **Conclusions:** MR-thermometry is an adequate method to precisely measuring in vivo deep brain thermal variations and allows thermal characterization of chronic implantable medical devices. The heat increase pattern was correlated to the light power delivered.

Disclosures: **C. Moro:** A. Employment/Salary (full or part-time): CEA-Leti/Clinatec. 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; Fondation France Parkinson. **J. Molet:** A. Employment/Salary (full or part-time): CEA-Leti/Clinatec. 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; EuroTalents/Carnot. **V. Auboiroux:** A. Employment/Salary (full or part-time): CEA-Leti / Clinatec. 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; Fondation France Parkinson. **F. Reinhart:** A. Employment/Salary (full or part-time): CEA-Leti / Clinatec. 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; Fondation France Parkinson. **S. Renault:** A. Employment/Salary (full or part-time): CEA-Leti / Clinatec. **C. Chabrol:** A. Employment/Salary (full or part-time): CEA-Leti / Clinatec. **N. Torres-Martinez:** A. Employment/Salary (full or part-time): CEA-Leti / Clinatec. **J. Mitrofanis:** A. Employment/Salary (full or part-time): Sydney University. **A. Benabid:** A. Employment/Salary (full or part-time): CEA-Leti / Clinatec.

Poster

755. Computational Tools: Outcomes and Evaluations

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 755.09/NNN34

Topic: I.06. Computation, Modeling, and Simulation

Title: Application of spatiotemporal variations of correlation dimension & nonlinear interdependence of scalp EEG for seizure prediction in pediatric myoclonic epilepsy

Authors: M. SHARIFI KOLARIJANI[1], S. AMIRSALARI[2], *M. RAZA[3];
[1]New Hearing Technologies Res. Ctr., [2]Dept. of Paediatrics, Fac. of Med., [3]Baqiyatallah Univ. of Med. Sci., Tehran, Iran, Islamic Republic of

Abstract: During the last two decades, several studies have shown that the information extracted from EEG recordings of epileptic patients can improve understanding of spatiotemporal dynamics of seizures. These studies have specifically investigated the possibility of seizure prediction using nonlinear time series analysis of EEG with evidence for the existence of a pre-

seizure state. In this study, we evaluated the predictive ability of two nonlinear measures of correlation dimension (CD) and nonlinear interdependence (NI) for seizures in pediatric myoclonic epilepsy patients using scalp EEG recordings. The sample contained EEG recordings of 11 patients (4 females with the average age of 10.3±4.6 yrs) using the 10-20 scheme or its extended version. The data was collected at Shifa Neuroscience Research Center, Khatam ul Anbia Hospital, Iran, during the period of 2010 to 2015. For each subject, one myoclonic seizure is analyzed. The exact onset of seizures was determined by a neurologist. The time profile of CD & NI were derived for each channel (pair) using a windowing technique for a 65 min EEG data (60 min before and 5 min after seizure onset) - for two patients, a portion of total 65 min data was available. For NI, only local combinations of channel pairs were included. Also, for each channel pair, the average of two NIs calculated for that pair was used as a symmetrized measure of NI. Finally, in a 60 min time interval before seizure onset, the amplitude distribution of CD and NI in inter-ictal period was compared to that of pre-ictal period using Receiver Operating Curve (ROC) analysis, for five different pre-ictal periods of 5, 10, 15, 20 and 25 min. The same analysis was performed comparing 2 & 5 min periods after seizures with 60 min period before seizure. The analyses were performed for two different schemes of all channels & individual channels. Furthermore, the spatiotemporal patterns of nonlinear measures in clinically relevant areas of the brain were compared with other areas for each patient. The results showed that these measures can differentiate the pre-ictal phase from the corresponding inter-ictal phase. However, there was no specific general behavior in either the timing or the direction (increase / decrease) of the pre-ictal change amongst patients, except for the observed increase in clinically relevant areas for NI in 70% of the cases. This observations indicate the importance of patient-wise tuning of any automated system for seizure prediction as well as suggest further studies for better evaluation of NI using scalp EEG in pediatric myoclonic epilepsy patients.

Disclosures: **M. Sharifi Kolarijani:** None. **S. Amirsalari:** None. **M. Raza:** None.

Poster

755. Computational Tools: Outcomes and Evaluations

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 755.10/NNN35

Topic: I.06. Computation, Modeling, and Simulation

Title: Lyapunov Exponent as a tool to evaluate the degree of neuronal insult in rat model of ischemic stroke

Authors: *S. PAUL[1], T. K. SINHA[1], R. PATNAIK[2];
[1]North-Eastern Hill Univ. (NEHU), Shillong, India; [2]Indian Inst. of Technol. (BHU), Varanasi, India

Abstract: Ischemic stroke is one of the enigmatic neurological disorders with least understood injuries. Electroencephalography (EEG) has been traditionally used to detect residual neural dysfunctions after cerebral ischemia but have several shortcomings yielding controversial and inconsistent results. This study involves lyapunov exponent analysis of electroencephalographic signals that can provide clear information regarding the long lasting neural impairment in the subjects suffered from ischemic injury. We have collected the EEG time series data of the fronto-parietal, occipital and temporal regions of three rat groups i.e. control, induced stroke and drug treated the rat brain. Lyapunov exponent is used to quantify the nonlinear chaotic dynamics of the signal. Furthermore, the distinct states of brain activity had different chaotic dynamics quantified by nonlinear invariant measures. It is generally estimated from the observed EEG time series data and that are used to confirm the chaotic nature of the EEG signals. The Lyapunov exponents are quantitative measure for distinguishing among the various types of orbits based upon their sensitive dependence on the initial conditions, and are used to determine the stability of any steady-state behavior, including chaotic solutions. Percentage recovery of signals from fronto parietal, occipital and temporal regions (all frequency bands) is analyzed. The lyapunov exponent was executed in Matlab platform. Our results clearly prove that Piroxicam is effective in reviving neuronal firing. This is the first ever finding which advocates the role of Piroxicam in neuronal firing apart from its other neuroprotective roles. Thus, the possibility of modulation of neuronal firing can act as a therapeutic strategy to prevent neuronal dysfunctions in cerebral ischemia.

Disclosures: **S. Paul:** None. **T.K. Sinha:** None. **R. Patnaik:** None.

Poster

755. Computational Tools: Outcomes and Evaluations

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Program#/Poster#: 755.11/NNN36

Topic: I.06. Computation, Modeling, and Simulation

Support: NIH Grant DC014701

NIH Grant DC014367

Title: Model definition and benchmarks for the Myriad parallel simulator

Authors: *P. RITTNER[1], T. A. CLELAND[2];
[1]Psychology, Cornell Univ. Dept. of Psychology, Ithaca, NY; [2]Psychology, Cornell Univ., Ithaca, NY

Abstract: Myriad is a compartmental biophysical simulator designed to parallelize even "densely integrated" neuronal and network simulations (in which many compartments affect one another at every time step) for high-performance execution on multicore CPUs or on nVIDIA GPUs under CUDA. Hence, large models can be designed with dense analogue interactions (e.g., graded synapses, gap junctions, mass diffusion) rather than being constrained to event-based coupling because of hardware limitations. This is accomplished via code generation techniques transparent to end users, who do not need to facilitate parallelization in their model descriptions. Specifically, models are dynamically translated into a custom-designed minimal object framework for C99 that runs natively on nVIDIA GPUs as well as on CPUs. Consequently, the same model can be executed on CPU or GPU via a compiler option, and models are fully thread-scalable to any number of available threads without user optimization. Dynamic parallelism on CPUs now is also supported via OpenMP, and asynchronous I/O support has been added to enable the concurrent streaming of data to storage, enabling simulations of indefinite duration. The C99 backend also has been refactored for greater efficiency. While the implementation layer is in C99 (with a custom-designed minimal object framework), users can design their Myriad models in any Python environment. Interface and display functions can use any Python-compatible frameworks and tools, whereas model design *per se* utilizes a subset of Python established as Myriad's domain-specific model description language (DSL). Within these DSL constraints, arbitrary user-defined functions and equations can be implemented. (Standard libraries will be provided for the established features of neural systems). The framework for high-level object construction in Python has been substantially expanded, and benchmarks for CPU and GPU simulations have been generated that demonstrate the efficiency of Myriad's architecture. We also discuss the potential for the Myriad backend to support other simulation frameworks.

Disclosures: **P. Rittner:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Equipment grant from NVIDIA Corporation. **T.A. Cleland:** None.

Poster

755. Computational Tools: Outcomes and Evaluations

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 755.12/NNN37

Topic: I.06. Computation, Modeling, and Simulation

Support: NIH grant R01DC009977

NIH grant T15LM007056

Title: Recent advances in ModelDB

Authors: *T. M. MORSE[1], R. A. MCDOUGAL[1], N. T. CARNEVALE[1], L. MARENCO[1,4,2], R. WANG[2,3], M. MIGLIORE[1,5], P. L. MILLER[4,2,3], G. M. SHEPHERD[1], M. L. HINES[1]; [1]Neurosci., [2]Ctr. for Med. Informatics, [3]Anesthesiol., Yale Univ. Sch. Med., New Haven, CT; [4]VA Connecticut Healthcare Syst., West Haven, CT; [5]Inst. of Biophysics, Natl. Res. Council, Palermo, CT

Abstract: Experiment-based models of neurons and neuronal circuits have grown increasingly complex since the early computational neuroscience work of Hodgkin and Huxley and Rall. Complexity potentially improves realism, but at the cost of reproducibility. ModelDB, founded in 1996, addresses this difficulty and enhances the scientific utility of computational neuroscience models by providing a convenient venue to share and discover model computer code associated with peer-reviewed publications. ModelDB now contains approximately 1100 published models covering more than 130 research topics built using a wide variety of simulation software. It is actively curated and developed to help users locate and understand models of interest. For example, every model entry is tagged with searchable metadata about the biological system and phenomena that it addresses. ModelDB also provides mechanisms to assist running models both locally and remotely, and has a graphical tool that enables users to explore the anatomical and biophysical properties that are represented in hundreds of the model entries. Each of its capabilities, from the model entry submitting and editing forms that the modeling community uses, to the search engines used to find models, the metadata itself such as the curated list of neuron and cell names, as well as the topic keywords used to further describe the models, is undergoing continued refinement and improvement.

Large research groups (Allen Brain Institute, Human Brain Project, etc.) are emerging that collect data across multiple scales and integrate that data into complex and numerous models. To support this new modeling paradigm, we have developed scripts to facilitate importing a large number of models. In the case of the Allen Institute models, our scripts produced a pure-NEURON version that is shared in addition to the original version which uses the Allen Institute's Software Development Kit. By using a semi-automated import process, not only are we able to reduce curation time, but we improve consistency and quality: the quality improves because advanced features like interactive results graphs for the readme can be developed once and used for all, making a low per-model investment in feature support. We are developing strategies to ensure that models from these large groups are individually discoverable while simultaneously ensuring that they do not impair the discovery of the contributions of other researchers.

Disclosures: **T.M. Morse:** None. **R.A. McDougal:** None. **N.T. Carnevale:** None. **L. Marenco:** None. **R. Wang:** None. **M. Migliore:** None. **P.L. Miller:** None. **G.M. Shepherd:** None. **M.L. Hines:** None.

Poster

755. Computational Tools: Outcomes and Evaluations

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Topic: I.06. Computation, Modeling, and Simulation

Support: NIH Grant R01 DC009977

NIH Grant T15 LM007056

Title: Automated metadata identification for better model discovery

Authors: *G. M. SHEPHERD, T. M. MORSE, R. A. MCDOUGAL;
Dept. Neurosci., Yale Univ. Sch. of Med., New Haven, CT

Abstract: Computational methods are now firmly embedded in neuroscience as techniques for extending experimental observations into predictive models. The ModelDB repository, home to over 1000 published neuroscience models, is a resource for sharing code, reducing the work and risk for creating new models. With the growth of ModelDB, it has become increasingly important that researchers can submit and locate models as efficiently as possible. To aid model discovery, submitters are asked to provide categorized metadata (cell type, brain region, ion channels, etc) describing their model. Unfortunately, this approach imposes a time burden on the submitter, and it risks an incomplete set of metadata due to misunderstandings or insufficient time. The ModelDB curator can also provide this metadata, but that shifts rather than eliminates the difficulty. To reduce the metadata entry time and to increase metadata completeness in both new and existing models, we have developed a tool that automatically makes suggestions for ModelDB metadata based on the abstract of a model's accompanying paper. Of the ModelDB abstracts we have tested as of our survey date, there were 1353 two-word phrases, 203 three-word phrases, 17 four word phrases, and 1 five word phrase appearing in the abstracts for at least five model papers. Repeated phrases like these often matched to neuroscience concepts. For example, the most common four word phrases were "spike timing dependent plasticity", "long term potentiation ltp", "timing dependent plasticity stdp", "cell patch clamp recording", and "deep brain stimulation dbs". We augmented this list of phrases with phrases identified as statistically correlated with given database metadata concepts and those identified by manual review of abstracts. Our suggestion tool uses generalized regular expression rules developed from this list of phrases, where the rules map generalized regular expressions to ModelDB metadata terms. Using this tool we were able to improve the completion of the existing ModelDB metadata fields, which will enable support for an increased number of searchable identifiers. This tool may have general applicability to enhancing searches for relevant metadata in neuroscience publications.

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Poster

755. Computational Tools: Outcomes and Evaluations

Location: Halls B-H

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Program#/Poster#: 755.14/NNN39

Topic: I.06. Computation, Modeling, and Simulation

Title: Computer-aided skeleton analysis of microglia de-ramification after experimental diffuse brain injury.

Authors: H. MORRISON[1], K. YOUNG[1], M. QURESHI[2], *J. LIFSHITZ[3];
[1]Univ. of Arizona, Tucson, AZ; [2]Barrow Neurolog. Inst., Phoenix Children's Hosp., Pheonix, AZ; [3]Barrow Neurolog. Inst., Phoenix Children's Hosp., Phoenix, AZ

Abstract: Traumatic brain injury (TBI) impacts the lives of over 2.2 million children and adults each year. Of additional concern are those that suffer more mild TBI, often not seeking medical attention, and minimizing the epidemiology of TBI. After the mechanical forces injure the brain, cellular processes include hemorrhage, edema, and inflammation that go on to exacerbate cellular damage. Microglia, brain immune cells, are constitutively active and finely attuned to change in the microenvironment surrounding neurons and glia. A hyper-ramified morphology represents microglial surveillance transitioning in response to first signs of pathophysiology and injury. Severe focal TBI results in rapid microglia de-ramification and amoeboid morphologies proximal to the lesion. However, following experimental diffuse brain injury, a model of mild TBI, amoeboid microglia are rarely observed and instead microglial hyper-ramification, activation, and rod morphology are observed across cortical regions by day 7 post-injury. Our purpose here was to map the spatiotemporal changes in microglial ramification after diffuse brain injury using a computer-automated, quantitative skeleton analysis. Rats were subjected to midline fluid percussion injury and tissue was harvested at 1, 2, 7 or 28 days post-injury (DPI; n = 3 per time point). Microglia were visualized by Iba-1 immunohistochemistry and captured on 40x digital photomicrographs. Images were processed using ImageJ protocols to visualize all microglia soma and process as binary and then skeletonized representations prior to Analyze Skeleton plugin and morphology data collection (number of microglia process endpoints/cell and process length/cell). The number of microglia endpoints/cell were decreased by ~30% (p<0.05, vs sham) in the injury site and beneath the temporal ridge at 1, 2, 7, and 28 DPI; microglia endpoints/cell were decreased in remote cortex only at 28 DPI (p=0.03). Also, summed microglia process length/cell was decreased at 7 and 28 DPI in the injury site and beneath the temporal ridge (p <0.05). Summarizing all data, we reveal spatiotemporal change in microglia process

length/cell (2-way ANOVA region: $F_{(2, 24)} = 28.8$, $p < 0.0001$; time $F_{(3, 24)} = 4.9$, $p=0.008$, no interaction) and a regional change in the number of endpoints/cell (region $F_{(2, 24)} = 14.6$ $p < 0.0001$; time $F_{(3, 24)} = 2.4$ $p = 0.09$; no interaction). By applying a skeleton analysis method to quantify microglia ramification, we reveal that microglia de-ramification occurs 1 day post-injury and progresses to remote areas over a 28 day time course. Skeleton analysis provides a quantitative approach to evaluate anti-inflammatory therapeutic efficacy.

Disclosures: **H. Morrison:** None. **K. Young:** None. **M. Qureshi:** None. **J. Lifshitz:** None.

Poster

756. Data Analysis and Statistics: Neuronal networks

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Program#/Poster#: 756.01/NNN40

Topic: I.07. Data Analysis and Statistics

Support: the National Basic Research Program of China （973 Program） granted No.2015CB755603

Title: Brain-wide and fast reconstruction of sparsely distributed neuronal trees

Authors: ***H. ZHOU**, S. LI, T. QUAN, J. LI, A. LI, Y. LI, H. GONG, S. ZENG;
Wuhan Natl. Lab. for Optoelectronics, Huazhong Univ. of Sci. & Technol., Hubei, China

Abstract: Digital reconstructed neural circuit helps us acquire the structural information of brain, and have a better understanding of how brain works. Reconstruction of single neural tree is one of the most important steps to reconstruct digital neural circuit. With the progress of sparse labeling and imaging technology, we obtain whole mouse imaging datasets with sparsely distributed neurons at micron spatial resolution. These neuron imaging datasets show us the fine structure of single neuron eliminating the adjacent signals interference, which makes it possible to fast reconstruct single neuron at brain-wide scale. Considering the size of the original datasets is more than 10 TB, manual reconstruction of a single neuron is labor intensive and time-consuming. Here we report a novel method of single completed neuron reconstruction introducing machine learning and big data technology. Assisted by only a few human interventions, our method can reconstruct the sparsely distributed neuronal trees at brain-wide scale within several hours 10 times faster than manual reconstruction.

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Poster

756. Data Analysis and Statistics: Neuronal networks

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Topic: I.07. Data Analysis and Statistics

Support: Marie Curie Initial Training Network NETT, project 289146

Marie Curie European Joint Doctorate COSMOS, project 642563

Title: Leaders and followers: Quantifying consistency of synfire chain patterns in neuronal spike trains

Authors: *T. KREUZ[1], M. POFAHL[2], E. RÄISÄNEN[1], M. MULANSKY[1];
[1]Inst. For Complex Systems, Sesto Fiorentino, Italy; [2]Dept. of Epileptology, University of Bonn, Germany

Abstract: In spiking neuronal networks perfectly consistent repetitions of the same global propagation pattern are called a synfire chain. For any set of spike trains the questions arise how closely it resembles such a synfire chain and which are the spike trains that lead/follow. Here we address these questions and introduce an algorithm based on two new indicators (termed SPIKE-Order and Spike Train Order) that allows to sort multiple spike trains from leader to follower and to quantify the consistency of the leader-follower relationships for both the original and the optimized sorting.
We illustrate our new method using spike train recordings from a neuronal network exhibiting Giant Depolarized Potentials. In the first analysis step we apply the adaptive coincidence detection originally proposed for the bivariate measure event synchronization [1] to act as match maker and to pair spikes such that each spike is matched with at most one spike in each of the other spike trains. We assign two bivariate order indicators to each spike. The SPIKE-Order distinguishes leading and following spikes, whereas the Spike Train Order evaluates the order of spike trains and allows to sort spike trains from leaders to followers. From these indicators we can define the Synfire Indicator which quantifies to what degree the unsorted spike trains resemble a perfect synfire chain. Since this Synfire Indicator is dependent on the order of spike trains we can search the permutation space of all spike trains to search for the order which results in the highest Synfire Indicator for the sorted spike trains. In a final step we evaluate the statistical significance of the results using a set of carefully constructed spike train surrogates. The algorithm is distinguished by conceptual simplicity, flexibility, low computational cost, and universality (parameter-free and time-scale adaptive). While here we present an application to neuronal spike trains, the algorithm is very generic and applicable to any kind of discrete data. Together with previous measures ISI-distance, SPIKE- distance and SPIKE Synchronization [2,3], SPIKE-Order is implemented in both the Matlab-based graphical user interface SPIKY and

the Python library PySpike [4].
1. Quian Quiroga R, Kreuz T, Grassberger P. Phys Rev E 66, 041904 (2002).
2. Kreuz T, Mulansky M, Bozanic N. JNeurophysiol 113, 3432 (2015).
3. Mulansky M, Bozanic N, Sburlea A, Kreuz T. IEEE Event-based Control, Communication, and Signal Processing 1 (2015)
4. Source codes of SPIKY and PySpike are available at http://www.fi.isc.cnr.it/users/thomas.kreuz/sourcecode.html and https://github.com/mariomulansky/PySpike, respectively.

Disclosures: **T. Kreuz:** None. **M. Pofahl:** None. **E. Räisänen:** None. **M. Mulansky:** None.

Poster

756. Data Analysis and Statistics: Neuronal networks

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Topic: I.07. Data Analysis and Statistics

Support: Wellcome Trust PhD studentship

NIHR Cambridge Biomedical Research Centre studentship

Title: Combined electrophysiological and morphological classification of neuronal cell types

Authors: *E. COTTERILL, S. J. EGLEN;
DAMTP, Univ. of Cambridge, Cambridge, United Kingdom

Abstract: Comprehensive classification of the numerous categories of neuronal cell types is an essential step in understanding the composition and functional properties of neuronal networks, as well as allowing for the precise manipulation of cellular activity using optogenetic techniques. Recently, several studies have examined the efficacy of using a combined data set consisting of both the morphological and electrophysiological properties of neurons to perform computational classification of cell types, and have come to conflicting conclusions about the value of utilizing both feature types for classification.
In the present study, we use data from the Allen Brain Atlas cell types database, which provides both electrophysiological recordings and reconstructions of a large number of cells in the mouse visual cortex, and morphological features calculated using the L-Measure software. We examined the impact of using this combined morphological and electrophysiological data set to classify neuronal cell types, and the level of class refinement that can be achieved by employing this combined feature set compared to a single feature type.

Results so far suggest that both the morphological and electrophysiological features provide important structure in this data set. These features do not produce a clear separation of cells by cortical layer, however, using Ward's hierarchical clustering method on the combined feature set, we can reliably differentiate between excitatory and inhibitory cells in the mouse primary visual cortex with over 90% accuracy (n=140 cells). Our analyses also show that strong correlations exist between certain morphological and electrophysiological features, and we explore which electrophysiological features are most tightly constrained by the underlying anatomical properties of a neuron.

Disclosures: E. Cotterill: None. **S.J. Eglen:** None.

Poster

756. Data Analysis and Statistics: Neuronal networks

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Topic: I.07. Data Analysis and Statistics

Support: MH068012

MH057153

NS011862

NS044820

Title: Neural network hidden layer units may model cortical neuron responses

Authors: *D. GARDNER[1], E. P. GARDNER[2];
[1]Physiol. & Biophysics, Weill Cornell Med. Col., New York, NY; [2]Neurosci. and Physiol., NYU Sch. of Med., New York, NY

Abstract: Connectionist or 'deep learning' neural networks, substrates for classifiers in both neural and artificial systems (Gardner, D. 1993, *The Neurobiology of Neural Networks*, MIT Press), are characterized by hidden layers. These leverage all-to-all or random connectivity and synaptic learning rules to instantiate intermediary representations of stimuli, serving as essential links between primary sensory information and object- or place-based or salient recognition and perception.
Both cortical and subcortical processing of sensory information may include distributed processing similar to that performed by hidden layers and units of such artificial neural networks. In 1989, neural network models were invoked to represent both transformation (Bankman,

Johnson and Hsiao) and plasticity (Grajski and Merzenech) in somatosensory systems. Subsequently, the neural network paradigm has been applied to sensorimotor behavior (Fetz 1993), magnetic fields evoked by somatosensory stimulation (Mauguiere et al 1997), the gate control theory (Melzack 1999), stationary vs. moving stimuli (Roy and Alloway 1999), and somatosensory working memory (Miller, Brody, Romo and Wang 2003).
Application of the neural network model may thus aid analysis of sensory representations. A parallel sensory system in *Drosophila* confirms the cross-phyla and cross-modality relevance of neuronal hidden layers. Here, Kenyon cells (KC) form a large hidden layer between input olfactory receptors and mushroom body output neurons (MBON). Caron, Ruta, Abbott and Axel (2013) reported that KCs receive random inputs from olfactory and odor-coded sensory glomeruli; they note this scheme is well suited for imposing learned valence on odors that lack innate hard-wired behavioral responses. Aso et al (2014) explicitly described a 'multi-layer feedforward readout network' of MBONs, extended by Hige et al (2015a,b).
Informing mammalian vision, Yamins and DiCarlo (2016) reviewed evidence that hierarchical convolutional networks, trained for visual object categorization, evolve firing patterns like cortical V4 and IT neurons.
Supporting tests of the relevance of this hidden-layer model for sensory processing, we offer STAToolkit, a set of information-theoretic tools (Goldberg, D.H. et al 2009, *Neuroinformatics* 7: 165 - 178). STAToolkit analyses of spike trains can reveal if these are consistent with a view of cortical neurons as hidden distributed-processing units that also incorporate biological variability.

Disclosures: D. Gardner: None. **E.P. Gardner:** None.

Poster

756. Data Analysis and Statistics: Neuronal networks

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Program#/Poster#: 756.05/NNN44

Topic: I.07. Data Analysis and Statistics

Support: Marie Curie Initial Training Network NETT, project 289146

Marie Curie European Joint Doctorate COSMOS, project 642563

Title: Measures of spike train synchrony: Defining a minimum time scale for the ISI- and the SPIKE-distance

Authors: *E. A. RÄISÄNEN[1,2], M. MULANSKY[2], T. KREUZ[2];
[1]Univ. of Florence, Sesto Fiorentino, Italy; [2]Inst. for complex systems, CNR, Sesto Fiorentino, Italy

Abstract: Over the years many different methods have been developed in order to quantify similarity of spike trains. For example, the Victor-Purpura [1] and van Rossum [2] metrics describe spike train (dis)similarity based on user-given time scales. The main drawback of these measures is the fixed time scale, since it sets a boundary between rate and time coding for the whole recording. This means that the result depends on the user input and the measures do not perform well if the spike trains contain more than one time scale.
These drawbacks have been eliminated in the time scale independent ISI-distance [3] and SPIKE-distance [4] by Kreuz et al., since these methods adapt to the local firing rate. However, while they identify correctly the relative firing rate differences, they have no concept of actual time scales. Especially in the presence of bursts this may lead to situations, where the time scale independence identifies small deviations from perfect synchrony as highly dissimilar.
Here we propose an extension to the existing ISI-distance and SPIKE-distance that is based on using a minimum relevant time scale (MRTS). This extension starts to gradually ignore differences in ISI that are smaller than the MRTS. The MRTS can be a parameter, but we also introduce a method for estimating it directly from the data. We perform a pairwise analysis on a library of stereotypical spike trains to show that the correction addresses the shortcomings of the original measures without introducing any side effects.
In summary, our new extension allows for a more accurate estimation of similarity with certain types of neuronal data. Especially in the presence of bursts the new version has the advantage of being able to disregard the differences that are too small for the time scales of the underlying system. Together with another new measure SPIKE Synchronization [5,6], all measures are implemented in both the Matlab-based graphical user interface SPIKY [7] and the Python library PySpike [8].
[1] Victor JD and Purpura KP. Journal of Neurophysiology 76(2):1310 (1996)
[2] van Rossum M. Neural Computation 13(4):751 (2001)
[3] Kreuz T, Haas JS, Morelli A, Abarbanel HDI, Politi A. J Neurosci Methods 165, 151 (2007)
[4] Kreuz T, Chicharro D, Houghton C, Andrzejak RG, Mormann F. J Neurophysiol 109, 1457 (2013)
[5] Kreuz T, Mulansky M, Bozanic N. JNeurophysiol 113, 3432 (2015).
[6] Mulansky M, Bozanic N, Sburlea A, Kreuz T. IEEE Proceeding on Event-based Control, Communication, and Signal Processing 1 (2015).
[7] http://www.fi.isc.cnr.it/users/thomas.kreuz/sourcecode.html
[8] https://github.com/mariomulansky/PySpike

Disclosures: E.A. Räisänen: None. **M. Mulansky:** None. **T. Kreuz:** None.

Poster

756. Data Analysis and Statistics: Neuronal networks

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Topic: I.07. Data Analysis and Statistics

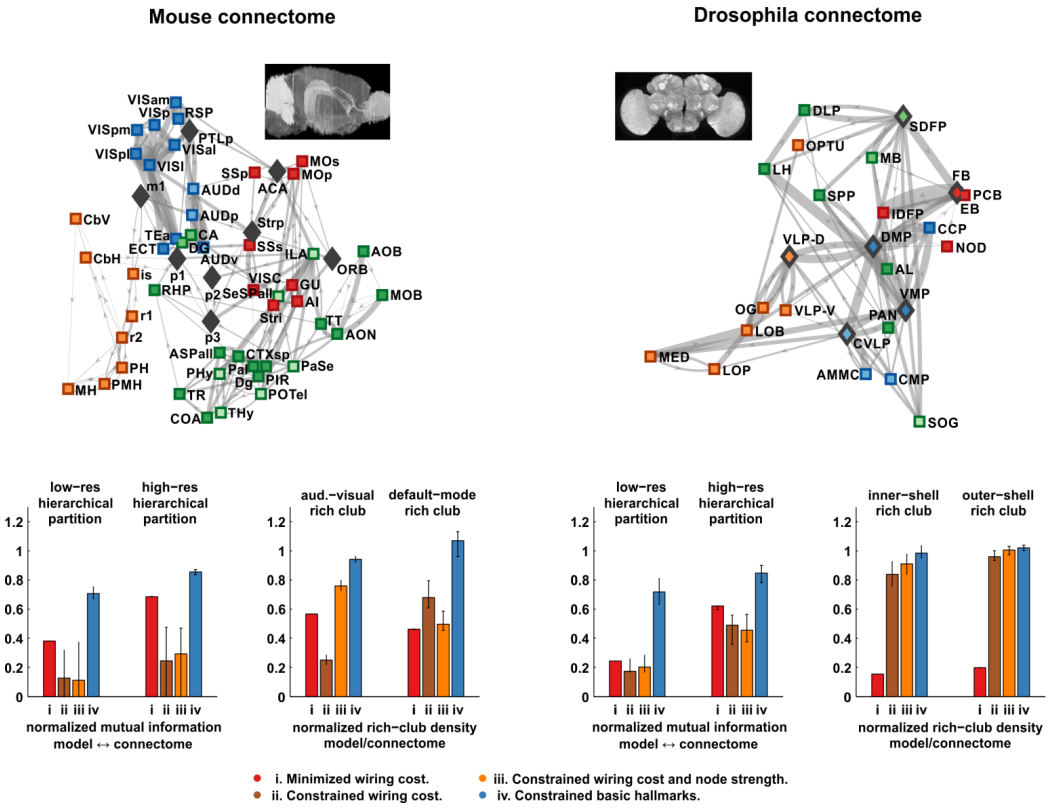
Support: NARSAD Young Investigator Grant 19490

Isaac Newton Trust Grant for Research Purposes 13.07(q)

Title: Constraints and spandrels of inter-areal connectomes

Authors: *M. RUBINOV[1,2];
[1]Janelia Res. Campus, Howard Hughes Med. Inst., Ashburn, VA; [2]Dept. of Psychiatry, Univ. of Cambridge, Cambridge, United Kingdom

Abstract: Understanding the principles of connectome organization is a grand challenge in systems neuroscience. Recently, module hierarchies and rich clubs have been described as two such organizational principles. Module hierarchies are nestings of smaller modules within larger modules, and are thought to enable complex brain dynamics by facilitating diverse functional states. Rich clubs are densely intra-connected groups of hub areas, and are thought to provide a backbone of functional integration. The prevailing paradigm underlying these discoveries, the cost-efficiency paradigm, postulates that hierarchies and rich clubs arise from competing pressures for wiring cost and information processing efficiency, in contrast to connectome modules, which arise primarily from wiring-cost pressures. Here we describe constraint network modeling, a distinct approach to the study of connectome organization. Our approach is based on constraining empirically observed - and therefore *a priori* biologically valid - connectome features, and on the study of the effect of these constraints on connectome organization. The presence of higher-order, not explicitly constrained, features in constraint network models, implies that such higher-order features are structural by-products, also known as spandrels, of the *a priori* specified constraints. Our results challenge the cost-efficiency paradigm of connectome organization in current gold-standard mouse and Drosophila inter-areal connectome reconstructions. Firstly, the results refute the determinacy of wiring-cost constraints on module organization, and show that hierarchies and rich clubs are structural by-products or spandrels of basic connectome organizational features. Secondly, the results imply that the currently standard literature reports of simultaneously present connectome modules, hubs, hierarchies and rich clubs are based on circular analyses. Finally, the generality and biological validity of constraint network models allows the future study of connectome organization in a more direct and principled way.



Disclosures: M. Rubinov: None.

Poster

756. Data Analysis and Statistics: Neuronal networks

Location: Halls B-H

Time: Wednesday, November 16, 2016, 8:00 AM - 12:00 PM

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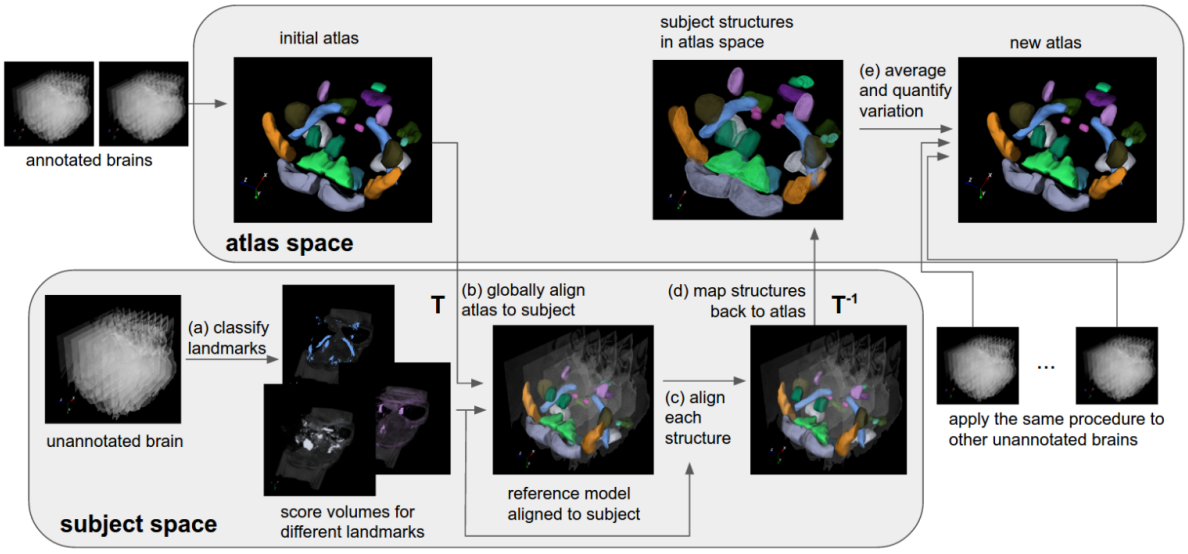
Title: Building an active digital atlas for the mouse brainstem

Authors: ***Y. CHEN**[1], A. TOLPYGO[3], D. FERRANTE[3], P. MITRA[3], M. GOULDING[4], H. KARTEN[2], D. KLEINFELD[2], Y. FREUND[1];
[1]Dept. of Computer Sci., [2]Dept. of Physics, UCSD, San Diego, CA; [3]Cold Spring Harbor Lab., Cold Spring Harbor, CA; [4]Salk Inst. for Biol. Studies, La Jolla, CA

Abstract: Anatomical maps play an essential role in the deconstruction of neuronal circuits. We aim at creating a digital atlas, with near-term focus on the mouse brainstem. The digital atlas allows us to form the mean position of brainstem nuclei, defining the notion of an average brain and the variations about it. This is essential as a framework to compile anatomical and functional measurements across experiments and experimental groups, including tracing studies of neuronal connections, electrophysiological recording studies of the action of neurons in different areas, and anatomical studies of different cell types. Further, the digital atlas is a guide to stereotaxic surgery and the placement of brain activity probes. Our atlas is an active atlas. Unlike a traditional graphic atlas, an active atlas is a software system that automatically maps image series of a brain to a standard coordinate system. It provides an anatomical reference model with position statistics of major structures computed from many subjects. It also contains texture detectors for salient regions like nuclei and tracts, which are used as landmarks for registration. Most importantly, it is updatable from new data to improve the estimate of position statistics; by mapping with the reference model, structure annotations are automatically transferred to new images, augmenting training examples for landmark detectors. Our data-driven approach (figure) builds an active atlas starting with images of 11 Nissl-stained Cryo-Jane sectioned brains. The reference model is bootstrapped by three annotated brains. For a new brain, the images are rigidly aligned, detectors are then applied to the images to generate a set of score volumes for different structures, and these are used to compute a global alignment that maps the reference model to the subject volume. Each structure in the model is then separately adjusted to better fit the subject, resulting in automatic annotations. Data from animals with labeled cells and/or cell types are then mapped back to the atlas coordinate system, allowing the position statistics to be computed.



Disclosures: **Y. Chen:** None. **A. Tolpygo:** None. **D. Ferrante:** None. **P. Mitra:** None. **M. Goulding:** None. **H. Karten:** None. **D. Kleinfeld:** None. **Y. Freund:** None.

Poster

756. Data Analysis and Statistics: Neuronal networks

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Future Systems Healthcare Project of KAIST

Title: Spatio-temporal classification of neural activity patterns in dynamic brain imaging data

Authors: ***M. SONG**[1,2], M. KANG1[1], H. LEE[1], H. LEE[1], Y. JEONG[1,2], S.-B. PAIK[1,2]; [1]Dept. of Bio and Brain Engin, [2]Program of Brain and Cognitive Engin., KAIST, Daejeon, Korea, Republic of

Abstract: Neural activity patterns observed in dynamic brain imaging data hold the clue to the mechanism of information processing in the brain. However, quantitative analysis or classification of these patterns has been considered as a challenging task, due to the nonlinearity

and complexity of neural activity patterns. Here we suggest a novel method of pattern classification that can represent both geometric structure and dynamic propagation of neural activity. We show that our method can successfully classify activity patterns in various types of imaging data, such as voltage-sensitive dye imaging (VSDI) and intrinsic optical signals imaging (IOSI) data.
To describe respectively the geometric structure and the propagation dynamics of activity patterns, we defined the geometric and dynamic profiles of spatio-temporal activity pattern. The geometric profile represents the information of size, amplitude and contour shape of activity, while the dynamic profile provides the information about propagation trajectory and degree of dispersion of activity.
We hypothesized the geometric and dynamic profiles can successfully classify different aspects of activity patterns in experimental imaging data. To test this, we tried to categorize VSDI and IOSI data of whole brain activity from Alzheimer (AD) and normal mouse groups. Using our geometric and dynamic profile analysis, we could hierarchically cluster the activity patterns from the paired correlation between the profiles of single pattern. As a result, the activity patterns of AD and normal mouse group appeared significantly separated clusters. This result suggests that our classification method can readily distinguish different dynamics of complicated activity patterns.
Our method provides an intuitive description of complex activity patterns and enables us to classify dynamics of various brain states effectively, which is generally applicable to various types of brain imaging data.

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Poster

756. Data Analysis and Statistics: Neuronal networks

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Topic: I.07. Data Analysis and Statistics

Title: Estimating dynamic functional networks of larger neural populations

Authors: *C. DONNER[1,2], H. SHIMAZAKI[3];
[1]BCCN Berlin, Berlin, Germany; [2]Tech. Univ. Berlin, Berlin, Germany; [3]RIKEN Brain Sci. Inst., Wako, Japan

Abstract: Information in the brain is encoded in spiking activity of neural populations. However the number of activity patterns that a population can generate increases exponentially with the

number of neurons. The maximum entropy method provides a principled way to describe the population activity using a tractable amount of parameters. This method successfully explained stationary spiking activity of neural populations by using less features, such as spike-rates of individual neurons and interactions among pairs of neurons, than the number of possible activity patterns. Modeling activity of cortical circuitries in vivo, however, has been challenging because both, the spike-rates and interactions, can change according to sensory stimulation, behavior, or an internal state of the brain. To capture the non-stationary functional network activity, a state-space framework was suggested to model dynamics of the neural interactions (Shimazaki et al. PLOS Comp Biol, 2012). However, based on exact analysis, the method suffers from computational cost; therefore its application was limited to only ~15 neurons. Here we introduce multiple analytic approximation methods to the state-space model, and make it possible to estimate dynamic pairwise interactions of up to 30 neurons. More specifically, we applied the pseudolikelihood approximation to the neural interaction model, and combined it with the TAP mean-field or Bethe approximation methods to carry out sequential Bayesian estimation of the model parameters. We found that belief propagation algorithm finds the solution of the Bethe approximation fast, and the provided solution is more precise than the TAP method. However, the belief propagation method sometimes fails to converge to a unique solution. Here we propose a hybrid method in which we use an alternative approach, the concave convex procedure (CCCP) that guarantees convergence, when a solution was not found by belief propagation. We compare the performance of these methods using simulated data, and demonstrate applicability of the method to experimental data recorded from awake animals. In addition to the time-varying interactions, the method allows us to investigate dynamics of global properties of recorded networks, such as entropy or sparsity of the population activity.

Disclosures: **C. Donner:** None. **H. Shimazaki:** None.

Poster

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Young Investigator Award from the Brain and Behavioral Research Foundation

Alfred P. Sloan Foundation Fellowship

Title: Changes in spatiotemporal network dynamics during learning

Authors: ***Q. SKILLING**[1], N. OGJANOVSKI[2], S. ATON[2], M. ZOCHOWSKI[3];
[1]Program in Biophysics, [2]Dept. of Molecular, Cellular, and Developmental Biol., [3]Dept. of Physics and Program in Biophysics, Univ. of Michigan, Ann Arbor, MI

Abstract: In order to function properly, the brain must operate in a dynamical regime which facilitates appropriate behavioral response to incoming stimuli. Such spatiotemporal dynamics of the brain are often quantified as functional connections between neurons using various statistical metrics on the available neural activity. Additionally, many studies have shown that neural networks of the brain exhibit phase transitions in activity; at a critical point along this transition, neural activity arranges as neuronal avalanches, where the probability of observing large events of consecutive activity follows a power law relationship with specific slope. In this study, we investigate the correlation between functional connectivity motifs, network proximity to a critical point, and their collective relationship to learning behavior. Using a simulated attractor neural network based on the Hopfield formalism, we change the system proximity to a critical point by varying a control parameter and show that training the network to store a new memory causes specific changes in functional connectivity only when the system resides near a phase transition in activity. Further, we analyze the spatiotemporal dynamics of hippocampal CA1 activity in mice undergoing contextual fear conditioning (CFC). Mice experiencing CFC show an increase in contextual freezing behavior after a single training trial. We examine the functional connectivity of these biological neural systems using the average minimum distance (AMD) metric of detecting statistically relevant coincident spiking between neurons, then, using cosine similarity between these AMD functional connections, we measure stability of these functional representations. We also measure the changes in the avalanche statistics. We show that learning novel information on one hand stabilizes functional representations in the hippocampus while on the other hand increases the spread of spiking avalanches in the network. Taken together, these results indicate that contextual learning imposes specific changes on the spatiotemporal network dynamics, affecting the functional connectivity of hippocampal nodes as well as neural population avalanche distributions, both of which may be used as reliable indicators of expected biological function.

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Poster

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Topic: I.07. Data Analysis and Statistics

Support: NSERC, Alexander Graham Bell Canada Graduate Scholarship-Master's (CGS M)

Title: Structural and functional connectivity relationships in the brain: How do they vary between large-scale networks and across individuals?

Authors: *K. G. SOLAR[1], J. KORNELSEN[2], S. M. COURTNEY[3], C. R. FIGLEY[2];
[1]Physiol. and Pathophysiology, [2]Radiology, Univ. of Manitoba, Winnipeg, MB, Canada;
[3]Psychological and Brain Sci., Johns Hopkins Univ., Baltimore, MD

Abstract: Structural connectivity (SC) between two locations in the brain indicates that these locations have physical white matter tracts connecting them, as visualized using structural MRI. Functional connectivity (FC) between two locations in the brain indicates that these locations have temporally correlated neural activity, as visualized by fMRI. Brain regions with FC are organized into large-scale networks that are involved in daily tasks. Data-driven examinations of SC-FC relationships in the brain have revealed that direct SC is generally predictive of FC, but that FC is not necessarily predictive of direct SC. These results suggest that SC-FC relationships are not one-to-one but highly complex. In order to elucidate the SC-FC relationships within brain networks, we conducted novel tests of these relationships using a hypothesis-driven, region-of-interest (ROI) approach. To do so, we employed the recently reported UManitoba-JHU Functionally-Defined Human White Matter Atlas, which provides group probability maps of white matter regions underlying six resting state networks, including the dorsal and ventral default mode, left and right executive control, and anterior and posterior salience networks. The current study utilized 3T MRI data (diffusion tensor imaging [DTI]; Myelin Water Imaging [MWI]; and resting-state fMRI) from 32 neurologically-healthy adults. For SC, subject mean diffusivity (MD) and fractional anisotropy (FA) maps were calculated from the DTI data, and myelin water fraction (MWF) maps were calculated from the MWI data. Using these novel atlases, we then created maps of each ROI-to-ROI combination in each network that allowed us to extract the corresponding FA, MD and MWF values for each combination. For FC, a pre-existing functional atlas was used to extract the corresponding FC values from the same ROI combinations, based on spontaneous blood oxygen level-dependent changes as measured using resting-state fMRI. For SC-FC relationships, we tested whether each SC measure (FA; MD; MWF) was correlated with FC between the same functional regions and across subjects. Certain ROI-to-ROI combinations in each network were identified as having strong SC-FC correlations, and patterns emerged among the most highly correlated regions within each network, suggesting their potential roles as network hubs. Finally, we established normal SC-FC relationships within these networks, thus allowing us to establish a baseline for future clinical studies involving abnormal relationships in patient populations.

Disclosures: **K.G. Solar:** None. **J. Kornelsen:** None. **S.M. Courtney:** None. **C.R. Figley:** None.

Poster

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PNI Innovation Award to SK

NJCBIR CBIR11PIL026 to MP

Title: Diffusion MRI in the non-human primate at 3T: a direct comparison of multi- vs. single-shell acquisitions

Authors: *M. A. PINSK[1], S. KASTNER[1,2];
[1]Princeton Neurosci. Inst., [2]Dept. of Psychology, Princeton Univ., Princeton, NJ

Abstract: In recent years diffusion MRI (dMRI) studies have advanced from relatively few number of gradient directions acquired with a single diffusion weighting to more complex multi-shell acquisition schemes and reconstruction methods (Tuch 2004; Tournier et al. 2004; Behrens et al. 2007; Wedeen et al. 2008; Aganj et al. 2010; Jbabdi et al. 2012). Here we asked whether a multi-shell scheme can improve accuracy of tractography through complex white matter architecture in the *in-vivo* non-human primate (NHP) model compared to a single-shell scheme with a similar density coverage and acquisition time.
Initial datasets were acquired at a Siemens MAGNETOM 3T Prisma scanner in the same animal under anesthesia with a 5-channel transmit/receive coil system using single-shell and 3-shell acquisition schemes (270 directions, b= 1000 s/mm^2 versus 90 unique directions per shell, b= 1000, 2000, 3000 s/mm^2). All other acquisition parameters were kept identical (TR= 9500ms; TE= 86ms; IPAT= 2; in-plane resolution= 0.8mm; slice thickness= 0.8mm). Recommendations provided by the Human Connectome Project (Sotiropoulos et al. 2013) were followed closely (e.g. interleaved acquisition schemes, polarity reversed slice select gradients for fat suppression, left-right and right-left phase encode directions to eliminate susceptibility distortions, monopolar diffusion encoding to reduce TE). Data were acquired twice under reversed phase-encoding to correct for susceptibility-induced distortions with FSL's topup tool (Andersson et al. 2003), and corrected for eddy current-induced distortions with FSL's eddy tool (Andersson & Sotiropoulos 2016). Both datasets were reconstructed with FSL's bedpostx tool ("zeppelin" axially symmetric tensor model with 3 fiber populations per voxel) (Sotiropoulos et al. 2015 ISMRM). Reconstructed datasets were imported to DSI-Studio (http://dsi-studio.labsolver.org) for

streamline tractography or FSL's probtrackx for probabilistic tractography.
Preliminary results suggest that the reconstructions of both the high-density single-shell dataset and the multi-shell dataset successfully modeled voxels with 2-fiber crossings throughout much of the white matter. However the multi-shell scheme allowed for detection of more voxels whose diffusion signal could be accounted for by 3 fiber orientations. Most of these voxels were found, as expected, in the centrum semiovale of the white matter, but remained quite sparse. Future plans include performance of tractography through the region as well as further improvements in acquisition (e.g. denser multi-shell coverage over longer periods of scan time).

Disclosures: M.A. Pinsk: None. **S. Kastner:** None.

Poster

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Topic: I.07. Data Analysis and Statistics

Support: NIMH RO1-MH064537

Title: Detecting multivariate cross-correlation structure between brain regions

Authors: *J. RODU, N. KLEIN, Y. YANG, E. AMINOFF, M. J. TARR, R. E. KASS; Carnegie Mellon Univ., Pittsburgh, PA

Abstract: When recordings from multiple electrode arrays, ECoG, EEG, or MEG are used to establish functional connectivity across two or more brain regions, multiple signals within each brain region must be combined. If, for example, signals across multiple electrode arrays in each of two regions are examined, the problem is to describe the multivariate relationship between all the signals from the first region and all the signals from the second region, as it evolves across time, during a task. While it is possible to take averages across signals, or across numerical summaries of pairwise interactions, this averaging may lose important information. We have investigated an alternative approach, which is capable of finding multivariate interactions among signals that are highly non-stationary due to stimulus or behavioral effects. For a single stationary time series in each of two brain areas statistical tools such as cross-correlation and Granger causality may be applied. On the other hand, to examine multivariate interactions at a single time point, canonical correlation, which finds the linear combinations of signals that maximize the correlation, may be used. However, an implementation of vanilla canonical correlation analysis to multivariate signals across time points inevitably fails to regard time as a special variable. The method we have developed produces interpretations much like these

standard techniques and, in addition, (a) extends the idea of canonical correlation to 3-way arrays with one dimension indexed by time (and the others by number of signals given and number of trials), (b) allows for non-stationarity, (c) also allows for nonlinearity, (d) scales well as the number of signals increases, and (e) captures predictive relationships, as is done with Granger causality. We demonstrate the effectiveness of the method through simulation studies and illustrate with MEG data.

Disclosures: **J. Rodu:** None. **N. Klein:** None. **Y. Yang:** None. **E. Aminoff:** None. **M.J. Tarr:** None. **R.E. Kass:** None.

Poster

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Topic: I.07. Data Analysis and Statistics

Support: AMED Japan Brain/Minds

MEXT Japan KAKENHI 16K16121

Title: Detection of predictive spike patterns with products of generalised linear models

Authors: ***T. HAYAKAWA**, T. FUKAI;
RIKEN BSI, Wako Saitama, Japan

Abstract: How neuronal circuits encode information into spikes has been a central question in neuroscience. It has been hypothesized that not only firing rates of single neurons but also spatiotemporal spike patterns possess information. Experimental studies have reported repeatedly observed spike patterns both in vitro and vivo. Computational studies have proposed candidate mechanisms underlying those spike patterns. The statistical significance of those spike patterns has often been quite controversial, however, and their functional role is still elusive. This is partly because there is a technical difficulty in identifying a combination of spikes whose coincidence has a large predictive value. This difficulty arises from the fact that the number of candidate combinations grows exponentially with respect to the number of the observed neurons and the length of the examined time interval.To address this issue, we consider products of generalised linear models. In this model, each basis function represents a predictive value of a spike of a specific neuron at a specific timing and hence products of these functions represent that of coincident spikes. Although estimation of parameters in this model is a highly non-convex problem, we derive a convex algorithm for this estimation. Then, under a suitable

assumption of sparseness, we prove that the estimated values of the parameters converge to the optimal values as the amount of the observed spike train is increased. We also show numerically that the proposed algorithm successfully detects predictive spike combinations.

Disclosures: **T. Hayakawa:** None. **T. Fukai:** None.

Poster

756. Data Analysis and Statistics: Neuronal networks

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Topic: I.07. Data Analysis and Statistics

Title: Quantifying network structure during state changes in functional brain networks

Authors: ***M. VAIANA**[1], S. E. F. MULDOON[2];
[1]Mathematics, Univ. At Buffalo, Amherst, NY; [2]Mathematics and CDSE Program, Univ. at Buffalo, Buffalo, NY

Abstract: Dynamic neural information can be encoded in functional brain networks but these dynamics can change with time, task, and brain state. Multilayer networks form a novel framework for encoding time dependent information and can be used to monitor and track evolving network structures. Recently it has been shown that community detection (or clustering) with a generalized multilayer modularity function is possible, but the results of such clustering are highly parameter dependent. Due to this sensitivity with respect to the parameters of the clustering algorithm, one must question how to optimize the choice of parameters for a given data set. This concern is particularly salient in data in which state changes are present, as would be expected in neural systems, since an abrupt change in system dynamics can result in a change in network structure. In order to address this concern, we use synthetic neural data to investigate the sensitivity of this method to the presence of noise and state changes in functional brain networks. We present results detailing the performance of the clustering algorithm with respect to the choice of parameters and provide recommendations for how to choose algorithm parameters to best represent community structure in noisy data in which state changes are present.

Disclosures: **M. Vaiana:** None. **S.E.F. Muldoon:** None.

Poster

756. Data Analysis and Statistics: Neuronal networks

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Topic: I.07. Data Analysis and Statistics

Title: Momentum: a new approach to signal detection

Authors: *N. Z. BIELCZYK, J. GLENNON, C. BECKMANN;
Donders Institute, Radboud Univ. Nijmegen Med., Nijmegen, Netherlands

Abstract: Gaussian white noise is highly prevalent in human environment, from telecommunication to synaptic transmission in the brain. Detection of signal against white noise is typically based on measures of non-Gaussianity such as skewness or kurtosis of the samples' distribution. These measures for non-Gaussianity are particularly useful in the context of Independent Component Analysis for fMRI data. The success in unraveling the ICA mixing matrix depends on the match between the type of non-Gaussianity incorporated in the ICA algorithm, and the non-Gaussianity in the data. In this work, we propose 'Momentum': a novel approach to non-Gaussianity, based on a combination of all central moments of the samples distribution. We argue that this is an efficient method for signal detection in case we have no prior information about the spectral properties of the signal. If we do not know the frequency content of the hypothetical signal, time order of the samples does not matter to the signal detection, and the only information which can be used to answer the question is the statistical properties of the samples' distribution. Furthermore, the Gaussian white noise has a *unique characteristics* in terms of the central moments. Let us assume the noise time series is normalized. Then, its first moment (the mean) is equal to zero, second moment (variance) is equal to one, third moment (skewness) is equal to 0 etc. All the higher central moments of the Gaussian distribution are also fixed and can be easily obtained analytically. Therefore, aggregating higher order central moments together as one measure of nonGaussianity (or, in other words, measure of aberrance from Gaussianity) can bring high discriminability for the noisy signal.
In order to evaluate the method against the other methods for signal detection, we sampled a few types of signals often encountered in the environment: (a) a number of single events (governed by a Poissonian process); (b) a train of on- and off- states (governed by a Poissonian process); (c) a sinus function; (d) a mixture of sinus functions of a few frequencies**.** For each signal in our testing set, we compared the results against the null distribution. Our results suggest that for the selection of tested signals, Momentum is the most sensitive detection method. We conclude that Momentum is a powerful method for the signal detection, and the optimal method in case we do not have a prior information about the power spectrum of the signal. It is not only a promising

improvement to the ICA algorithms, but can also have implications to a broad variety of disciplines, from telecommunication to seismology.

Disclosures: **N.Z. Bielczyk:** None. **J. Glennon:** None. **C. Beckmann:** None.

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Title: Light-sheet microscopy of brain vascular image enhancement based on gradient adjust with split bregman

Authors: H. HUI, X. LIANG, D. DONG, X. YANG, *J. TIAN;
Key Lab. of Mol. Imaging, CAS, Beijing City, China

Abstract: Light Sheet Microscopy (LSM) is a high-resolution fluorescence microscopic technique which enables to observe the mouse brain vascular network clearly with immunostaining. However, micro-vessels are stained with few fluorescence antibodies and their signals are much weaker than large vessels, which make micro-vessels unclear in LSM images. In this work, we developed a vascular image enhancement method to enhance micro-vessel details which should be useful for vessel statistics analysis. Since gradient describes the edge information of the vessel, the main contribution of our method is to increase the gradient values of the enhanced image to make the micro-vessels contrast. Meanwhile, an optimum problem whose solution was the final enhanced image with increased gradient values was formulated by designing an energy function. Our method contained two steps: 1) calculate the gradient image of LSM image, and then amplify high gradient values of the original image to enhance the vessel edge and suppress low gradient values to remove noises. Then we formulated a new L1-norm regularization optimization problem to find an image with the expected gradient while keeping the main structure information of the original image. 2) The split bregman iteration method was used to deal with the L1-norm regularization problem and generate the final enhanced image.

The main advantage of the split bregman method is that it has both fast convergence and low memory cost. In order to verify the effectiveness of our method, we applied our method to a series of mouse brain vascular images acquired from a commercial LSM system in our lab. The experimental results showed that our method could greatly enhance micro-vessel edges which were unclear in the original images.

Disclosures: **H. Hui:** None. **X. Liang:** None. **D. Dong:** None. **X. Yang:** None. **J. Tian:** None.

Poster

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Topic: I.07. Data Analysis and Statistics

Title: Hippocampal neural events predict ongoing brain-wide BOLD activity.

Authors: *M. BESSERVE[1,3], N. K. LOGOTHETIS[2,4];
[2]Cognitive Neurophysiol., [1]MPI for Biol. Cybernetics, Tuebingen, Germany; [3]MPI for Intelligent Systems, Tuebingen, Germany; [4]Ctr. for Imaging Sciences, Biomed. Imaging Inst., The Univ. of Manchester, Manchester, United Kingdom

Abstract: Transient Local Field Potential (LFP) activity exhibits a wide variety of patterns, reflecting local antagonistic or synergistic neural activity changes in the recorded structure. Among them, “events” with a characteristic time-frequency profile can be identified, which may occasionally reflect transient large-scale interactions with other brain structures. Such an event-related multistructure activity can be studied using concurrent fMRI and LFP recordings in an experimental design dubbed as Neural Event Triggered (NET)-fMRI (Logothetis et al, 2012). Recently we used NET-fMRI to describe the brainwide up and down modulation of neural activity associatiated with hippocampal ripples. Here we examine how much the BOLD changes associated with these events can describe the fMRI time series along the entire data acquisition period. To address this question, we develop a generative model of the ongoing neural activity including both hippocampal LFP recordings and BOLD signals in the whole brain.
The model was based on 6 types of oscillations detected in the hippocampus: Sharp-waves, ripples, gamma, beta, sigma and low frequency events. We first estimated the time course of the BOLD signature of neural events across brain structures by learning a dictionary of responses using the kSVD algorithm, and performed statistical analysis to extract significantly activated voxels for each neural event. Based on a convolutive model of the LFP-BOLD relationship, we corrected the effects of overlap between successive neural events on the BOLD response by estimating the autocorrelation function of the neural events and used it to obtain deconvolved

BOLD signatures, describing the contribution of a single event to the BOLD signal. Preliminary results on 3 sessions show the BOLD signature of each event can be well captured by two dictionaries elements: one with a short response latency (peak response at 2.6s) in a wide range of subcortical and cortical structures, and a long latency (peak response at 5.1s) response restricted to sensory and associative cortical areas. This model enables us to estimate the contribution of hippocampus-related activity to fMRI time series in the whole brain. We thus estimated the overall ongoing single trial fMRI activity averaged across all brain structures at each time point using the model and LFP event time stamps only. The average correlation coefficient between the true fMRI signal and the event based reconstruction was .313, showing that hippocampal neural event carry rich information about global brain dynamics and suggesting that global brain dynamics could in turn be used to infer electrical activity non-invasively.

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